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JOURNAL OF DAIRY SCIENCE

VOLUME XVIII SEPTEMBER, 1935 NUMBER 9

THE VITAMIN A CONTENT OF PASTURE PLANTS

III. ALFALFA (*MEDICAGO SATIVA* L.) AND SMOOTH BROME (*BROMUS INERMIS* LEYSS.) UNDER PASTURAGE CONDITIONS AND FED GREEN*

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I. W. SLATER, AND R. F. JOHNSON
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This paper is the third of a series from this station on the vitamin A content of pasture plants under pasturage conditions and fed green. Plants previously reported on are: white clover, Kentucky blue grass, timothy, and red top. In the first two papers the reasons for interest in the vitamin A content of pasture plants were discussed and representative literature on the subject was reviewed. Citations will be limited to literature pertinent to this report.

The two plants upon which the vitamin A content is herein reported are alfalfa and smooth brome grass. All the alfalfa and part of the brome were grown under irrigation at the Caldwell Substation of the Idaho Agricultural Experiment Station. Two groups of rats were fed brome grown without irrigation at Moscow. The alfalfa used was the Grimm variety.

EXPERIMENTAL PROCEDURE

Essentially the same procedure was used as in the two previous reports (3, 4). Vitamin D was supplied by three drops of Mead's Viosterol per week, as in the second report. Groups I to IV inclusive were fed alcohol-extracted casein as a vitamin A free protein source as in the first investigation (3) while groups V to VIII were fed heat-treated casein as reported in the second paper (4). Fresh green samples of alfalfa and brome were received twice a week. The samples of brome consisted mostly of medium sized leaves with very few stems. The alfalfa samples were leafy, but due to the rankness of growth considerable quantities of stems were present. Pasturage of each plant was typical of ordinary conditions. The experimental feeding period was April 18 to June 15 in 1932 and April 17 to June 12 in 1933.

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RESULTS

The average weight of the rats in all groups was 41 grams at the beginning of the depletion period and 110 grams at the end. The depletion period for all the rats averaged 31 days.

Table 1 and Figure 1 show the average growth response of the rats fed each of the pasture plants. Results are presented for periods of four weeks (28 days), and the rat units per gram are calculated from the daily dose in milligrams required to cause a gain of 12 grams in 4 weeks. All rats which had not attained a minimum gain of 12 grams in the 4-week period were excluded from the results for average performance as required for assaying U. S. P. standard cod liver oil (1). The rats thus excluded from the averages are indicated by the difference between "rats fed" and "rats used" in Table 1.

When 60 milligrams of alfalfa were fed, the average total gain per rat (9 rats) was 32 ± 2 grams, or 8 grams per week, which would indicate 269 ± 17 rat units of vitamin A activity per gram. Rats fed 180 milligrams per week made an average total gain of 55 ± 5 grams in four weeks.

In 1932 eight rats, litter mates of those receiving 60 milligrams of alfalfa, made an average total gain of 47 ± 3 grams in four weeks when fed 60 milligrams of brome grass from Caldwell. This would indicate a vitamin A value of 396 ± 27 units per gram as compared with 269 ± 17 for alfalfa. Similarly, 11 animals fed 180 milligrams per week made an average total gain of 63 grams. In 1933 additional tests with brome grass were made. Three rats receiving 60 milligrams of this grass from the substation at Caldwell made an average gain of 44 grams which was in agreement with the previous year's results with grass from the same source. In the same year brome grass grown at Moscow (non-irrigated) gave an average total gain of 34 ± 3 grams for eight rats when fed at a level of 60 milligrams per week.

The coefficient of variation in the group fed alfalfa (60 mg. per week) was 26 per cent, while it was 27 in the group fed the same level of brome grass, from the same source (Caldwell). The variation in both groups was less than that reported by Sherman and Burtis (2).

Statistical analysis of the results showed that a true difference existed between the Caldwell brome and the Moscow brome when each was fed at the rate of 60 milligrams per week. The difference was 114 rat units and the probable error of the difference was 26. Since three times the probable error of the difference ($3 \times 26 = 74$) was not equal to the difference (114), some difference not due to methods was obtained. This difference in favor of the Caldwell brome may have been due to loss of moisture in shipping, thereby resulting in a more concentrated product than in the case of the Moscow brome which was taken directly from the field to the laboratory.

Comparison of results obtained from Caldwell brome and Caldwell alfalfa, each fed at a level of 60 milligrams, showed a difference of 127 rat units in favor of brome. The probable error of the difference was 22 units.

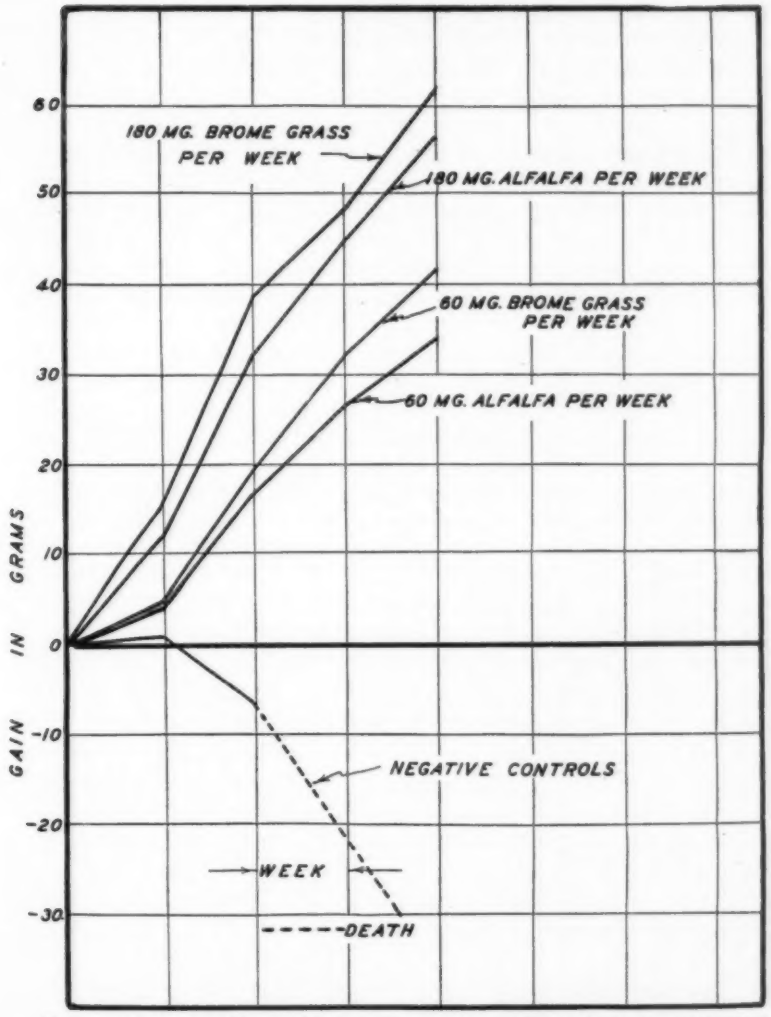

FIG. 1. Average weekly weight increases of rats, previously depleted of vitamin A, when fed either alfalfa or smooth brome as a supplement to a vitamin A free diet.

Average weekly losses in weight of negative control rats were computed each week by averaging the weight of all living rats as long as 50 per cent of the original group survived. The line was terminated by extending it directly from the last week showing 50 per cent survival to a point indicating the average survival days and average weight at death for the entire group. The broken line indicates that one or more deaths occurred during the week.

TABLE 1

Average growth response of rats when fed fresh alfalfa and brome grass

PASTURE PLANT	SOURCE	DATE OF TEST PERIOD	AMT. OF PLANT FED WEEKLY	RATS FED	RATS USED		AVE. WT. AT BEGINNING OF DEPLETION PERIOD	AVE. WT. AT BEGINNING OF TEST PERIOD	AVE. WT. AT END OF 4 WEEKS TEST PERIOD	AVE. GAIN IN WT. IN 4 WEEKS	COEFFICIENT OF VARIATION	RAT UNITS OF VITAMIN A
			Grams	No.	No.	%	Grams	Grams	Grams	Grams	%	
Alfalfa	Caldwell	4–18–32 to 6–15–32	0.060	10	9	90	41	116	148	32 ± 1.9	26	269 ± 17
Alfalfa	Caldwell	4–18–32 to 6–15–32	0.180	10	9	90	42	117	173	55 ± 4.8	36	154 ± 14
Brome Grass	Caldwell	4–18–32 to 6–15–32	0.060	8	8	100	41	116	163	47 ± 3.2	27	396 ± 27
"	"	"	0.180	11	11	100	41	117	180	62 ± 4.4	34	173 ± 12
"	"	4–17–33 to 6–12–33	0.060	3	3	100	40	104	148	44	*	*
"	Moscow	4–17–33 to 6– 9–33	0.060	8	8	100	41	100	134	34 ± 3.2	37	282 ± 27
Negative Controls				15			45	118	Ave. survival—25 grams Ave. loss in Wt.—30 grams			

* Insufficient data.

The difference was a true one, it being equal to more than 5½ times the probable error. Thus brome grass was superior to alfalfa as a source of vitamin A activity when fed at the same level. Observations of the test animals during the feeding period seemed to substantiate the superiority of brome when fed at the same level as alfalfa.

In order that the results obtained may be compared with data reported on the basis of 24 grams gain in eight weeks, the average gain made by each group in eight weeks is given. When the average gain for the longer period is the basis used for calculating the rat units per gram, all animals in a group are usually included whether or not they are gaining at the end of the test. All animals, therefore, are used in summarizing the eight-week averages.

When fed alfalfa at the rate of 60 milligrams per week, the animals (9 rats) made an average total gain of 42 ± 3 grams in eight weeks, indicating the vitamin A activity to be 175 ± 14 rat units per gram. Brome grass fed at the same rate produced an average total growth of 65 ± 5 grams, or 271 ± 20 rat units of vitamin A activity per gram.

The 15 animals used as negative controls survived an average of 25 days and lost an average of 30 grams in body weight. All rats had typical symptoms of vitamin A deficiency.

DISCUSSION

The six pasture plants which have been studied under pasturage conditions would rank as follows in vitamin A activity per gram: Smooth brome, 396 ± 27 rat units; red top, 308 ± 10; alfalfa, 269 ± 17; white clover, 242 ± 19; timothy, 220 ± 13; and Kentucky bluegrass, 175 ± 11. The high ranking of these plants compared with other foods and feeds as sources of vitamin A activity should again be emphasized.

It is difficult to attach any significance to the type of plant and its content of vitamin A activity. Alfalfa and white clover are legumes, and the other four plants are non-legumes. Both the highest and lowest ranking grasses are non-legumes. Other characteristics such as rankness of growth, size of leaf, depth of root, etc., do not appear to have any relationship to vitamin A activity of the plant.

Although no tests were conducted, it is probable that significant differences do exist in the carotene content of the various plants, which may in turn harmonize with the rank of the plants in content of vitamin A activity.

All the plants were assayed for vitamin A activity in the early spring and samples were taken from fields being pastured by dairy cows. Undoubtedly different results would be obtained if the plants had been allowed to become more mature without pasturage, as the plants differ considerably in the degree of green color at maturity. From the standpoint of the effect on

the vitamin A activity of butter it is important to study the plants under the same conditions as they are consumed by the cow in grazing.

CONCLUSIONS

The results indicate that when sampled under pasturage conditions Smooth brome grass (*Bromus inermis L.*) contained 396 ± 27 rat units of vitamin A activity per gram, and alfalfa (*Medicago sativa L.*) contained 269 ± 17 units. A rat unit as used in this paper is calculated from the daily dose in milligrams required to cause an average total gain of 12 grams in 4 weeks.

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THE CREATINE TEST FOR ACETYLMETHYLCARBINOL PLUS DIACETYL IN BUTTER CULTURES*

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The investigations of van Niel, Kluyver and Derx (8) and of Schmalfuss and Barthmeyer (7) established the importance of diacetyl as an aroma constituent of butter and suggested that the diacetyl is formed through the oxidation of acetylmethylcarbinol. Following these studies, the Iowa Agricultural Experiment Station (4) reported data showing that butter cultures having a satisfactory flavor and aroma contain relatively large amounts of acetylmethylcarbinol plus diacetyl, while butter cultures lacking in flavor and aroma contain comparatively small amounts or none of these materials.

The quantitative determination of acetylmethylcarbinol plus diacetyl in a butter culture usually involves the steam distillation of a portion of the culture, after adding ferric chloride to oxidize the acetylmethylcarbinol to diacetyl, and the precipitation of the distilled diacetyl (both that originally present and that formed from the acetylmethylcarbinol) as nickel dimethylglyoximate, which is filtered off and weighed. This procedure is time-consuming and can be carried out only with considerable laboratory equipment. A simple and rapid test which would permit an approximation of the amount of acetylmethylcarbinol plus diacetyl in a butter culture would be useful in comparing cultures from day to day and also in checking the cultures used in a plant or in a creamery organization.

In an investigation of the nature of the Voges-Proskauer reaction, Harden (1) showed that acetylmethylcarbinol, produced from glucose by certain organisms, is one of the necessary factors in the reaction and that some substance present in peptone water is also required. Harden and Norris (2), working with diacetyl, found that the reaction is dependent on the presence of some material, such as arginine or creatine, containing a certain atomic configuration. The importance of acetylmethylcarbinol in the Voges-Proskauer reaction and its formation by various organisms have centered attention on it as a product of bacterial metabolism, and many methods have been suggested for its rapid detection in bacterial cultures. O'Meara (5) considered these methods generally unsatisfactory and proposed growing the organism under investigation in a special medium and then testing for acetylmethylcarbinol by adding creatine and strong sodium hydroxide, shaking, allowing the mixture to stand, and examining for the appearance of a red color.

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The method which O'Meara employed seemed to be applicable to the rapid testing of butter cultures for acetylmethylcarbinol plus diacetyl and, accordingly, was tried for this purpose. The results obtained indicate that the method can be used to advantage in the examination of butter cultures.

The general procedure employed in the rapid examination of a butter culture for acetylmethylcarbinol plus diacetyl is as follows: Two and one-half cc. of the culture are poured into a test tube having an inside diameter of about 0.5 inch and a small amount of creatine (a portion of the powder equal to about one-third of the volume of a wheat grain) added; the creatine can best be handled on the point of a small knife. Strong sodium hydroxide (40 gm. made up to 100 cc. with water) equal in volume to the volume of culture is then poured into the tube, the mixture thoroughly shaken and allowed to stand. If relatively large amounts of acetylmethylcarbinol plus diacetyl are present in the culture, a red color develops at the surface of the mixture in a few minutes or less and soon extends to a depth of 0.25 inch or more. With smaller amounts of acetylmethylcarbinol plus diacetyl the red color develops more slowly and the colored portion is comparatively shallow. Cultures which are decidedly lacking in flavor and aroma may show no red color even after holding the test mixture for hours. When a strong reaction is secured, the material adhering to the wall of the tube above the surface of the liquid is strikingly red in color.

If a series of butter cultures is arranged, by a competent judge, in the order of the intensities of the desirable flavors and aromas and then tested with creatine and sodium hydroxide, the intensities are closely correlated with the rapidities and extents of the color reactions. The butter cultures in use in plants show all degrees of color development with the test and the results of the test are, in general, in agreement with the intensities of the flavors and aromas, as determined by a competent judge. Occasionally butter cultures are seen which show no red color with the test, and these are conspicuously lacking in flavor and aroma. Milk cultures of *Streptococcus lactis* commonly fail to show any red color. In general, more red color is secured with butter cultures made from milk to which citric acid has been added than with cultures made from unmodified milk.

The formation of only a thin red band on the surface of the test mixture should not be interpreted as indicating that the flavor and aroma of the culture are entirely satisfactory, since this may occur with cultures which yield only a relatively small amount of nickel dimethylglyoximate when the acetylmethylcarbinol plus diacetyl is determined quantitatively. A culture with a high flavor and aroma rapidly gives a rather broad band of red color.

The creatine test makes it possible to quickly secure general information on the comparative amounts of acetylmethylcarbinol plus diacetyl present in butter cultures. For the experienced judge such information is useful as a means of confirming the impressions obtained through the senses of taste

and smell, while for the inexperienced judge it can be used to help in developing the ability to properly evaluate cultures. When the method is to be employed in a plant or creamery organization, it should be standardized as well as possible by using (a) approximately the same amount of creatine, (b) sodium hydroxide of a uniform strength, (c) uniform volumes of culture and sodium hydroxide, etc. The importance of approximately the same amount of creatine in various tests is suggested by the fact that Walpole (9) utilized the color given in alkaline solution by creatine when a trace of diacetyl is added for the quantitative estimation of creatine in urine. Levine, Epstein, and Vaughn (3) combined creatine and potassium hydroxide as one solution (0.3 per cent creatine in 40 per cent potassium hydroxide) for use in the Voges-Proskauer test; the solution was found to have limited keeping qualities, and these were greatly influenced by the temperature of holding. In the studies on butter cultures a solution of 0.3 per cent creatine in 40 per cent sodium hydroxide (40 gm. made up to 100 cc. with water) was tried, but the solution seemed to keep poorly at summer temperatures. The mechanism of the Voges-Proskauer reaction and the diacetyl reaction for proteins has been studied recently by O'Meara (6).

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A COMPARISON OF METHODS OF DETECTING STREPTOCOCCI IN FRESHLY DRAWN MILK SAMPLES

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Recent investigations on the etiology of infectious bovine mastitis emphasize the importance of detecting streptococci in the diagnosis of the disease. Hucker (4) found that milk from a normal quarter free from fibrosis does not contain long-chained streptococci. Ernst, Schmidt-Hoensdorf and Schmidt (1) succeeded in producing typical streptococcal mastitis by introducing streptococci from mastitis milk into the udder by way of the teat canal. Minett, Stableforth and Edwards (8) and Plastridge, Anderson, White and Rettger (10) have shown that herds free from infectious streptococcal mastitis may be established by the use of periodic bacteriological tests and segregation of animals shedding streptococci of the type associated with infectious bovine mastitis. While other organisms, particularly staphylococci, are occasionally responsible for udder disturbances, the evidence at hand indicates that a fairly well defined species of streptococcus is the principal etiological agent to be considered in attempts to control infectious bovine mastitis.

The recognition of streptococci as the principal cause of infectious chronic mastitis increases the importance of methods of detecting the presence of these organisms in the udder of the cow. The standard nutrient agar plate commonly used in determining the bacterial content of milk is generally recognized as unsatisfactory for the detection of udder streptococci. As blood agar prepared with beef or veal infusion supplies the growth requirements of most streptococci and at the same time yields information on the characteristics of the strains isolated, blood agar has been used widely in detecting streptococci in milk samples. Three principal methods of inoculating blood agar plates have been used: namely, (1) streaking one or several loopfuls of whole milk over the surface of the medium, (2) streaking sediment from centrifuged portions of the sample over the surface of the medium, and (3) plating diluted portions of the sample in blood agar.

Microscopic examination of stained films of fresh samples and films prepared from sediment have also been widely used. While the finding of long chains of streptococci by these two methods is generally regarded as conclusive evidence of streptococcal mastitis, the work of Rosell (11), Hucker (5), Halverson, Cherrington and Hansen (3) and Minett, Stableforth and Edwards (7) and Edwards (2) shows that animals affected with chronic

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mastitis commonly fail to shed a sufficient number of streptococci to be detected by these methods.

Recently, particular attention has been given to the significance of the finding of long chains of cocci in incubated samples. Halverson, Cherrington and Hansen (3) regard the mere presence of long chains of streptococci in incubated samples insufficient evidence of streptococcal mastitis, unless correlated with other findings. Hucker, Trudell and Jennings (6) consider the microscopic examination of incubated milk the most effective method of detecting udder streptococci.

Studies on infectious bovine mastitis have been in progress at the Storrs Agricultural Experiment Station since 1926. During the past two years emphasis has been placed on methods of diagnosing infectious mastitis. As a part of this investigation, six different methods of detecting the presence of streptococci in freshly drawn milk samples were compared for the purpose of selecting the most efficient means of detecting shedders of streptococci which are capable of causing infectious bovine mastitis. A detailed account of the results of other tests employed in the diagnosis of mastitis will appear in a later publication.

METHODS

Various tests for evidence of mastitis were made on the animals in the Connecticut State College herd at intervals of two weeks over a period of several years. In two of the regular tests, six different methods of detecting streptococci were employed, in addition to the determination of the leucocyte count, sediment volume, reaction to the bromthymol blue test, and the appearance of the sample. The results given by the different methods, and the relation of the results to each other were considered with other evidence of mastistis before, at the time of, and after the tests were made.

Collection of Samples.—Before collecting the samples the lower portion of the udder and teats, including the opening of the teat canal, were washed with a solution containing 500 parts of chlorine per million parts of water. Badly soiled udders were given a preliminary washing with water. After discarding the first four streams, separate samples were collected from each quarter of the experimental animals.

Examination of Samples.—A detailed account of the methods used in the examination of the samples, and the basis of classifying animals as negative, suspicious or positive for mastitis, have already been reported in Storrs Experiment Station Bulletin 197 (10).

Detection of Streptococci.—A brief statement of the methods employed in detection of streptococci follow:

1. A four mm. loopful of the whole milk sample was streaked over the surface of a blood agar plate.

2. A 1 to 10 dilution of the sample was prepared and plated in blood agar, according to the method described in Standard Methods of Milk Analysis, Sixth Edition, page 64 (12).

3. A four mm. loopful of the material remaining in the conical end of a centrifuge tube after centrifuging a 10 cc. portion of the sample was streaked over the surface of a blood agar plate.

4. A direct microscopic examination was made of stained films of the fresh samples prepared according to the method of Breed. The presence or absence of cocci occurring in pairs or chains was noted.

5. Films prepared from the sediment obtained from centrifuged portions of the sample were also examined for the presence of streptococci.

6. A microscopic examination was made of stained films prepared from portions of samples which had been incubated from 18 to 20 hours at 37° C. The presence or absence of streptococci occurring in chains of ten or more cells was recorded.

Evidence of Mastitis.—Samples were classified as positive or negative for mastitis, on the basis of the appearance of the sample, sediment content, reaction to the bromthymol blue test and the leucocyte count. A detailed description of all possible combinations of results given by the tests employed is considered out of place here. In general, an abnormal appearance of the sample, more than 0.5 per cent by volume of sediment, the yielding of a distinctly abnormal color to the bromthymol blue test and a leucocyte count of over one million cells per cubic centimeter, were all regarded as evidence of mastitis. The results obtained on samples collected during a period of two weeks following parturition and during the last 30 days of the lactation period were not used in this study.

Results

Three hundred and sixty milk samples were examined for the presence of streptococci by the six methods described.

Blood Agar.—The number of samples yielding weakly hemolytic streptococcus-like colonies on blood agar inoculated by the three methods employed is given in Table 1.

As expected, blood agar plates inoculated by streaking a loopful of whole milk over the surface of the medium detected the presence of streptococci in fewer samples than the other two methods. Weakly hemolytic streptococcus-like colonies were found on 51 or 14.2 per cent of the plates streaked with whole milk, on 66 or 18.3 per cent of the plates streaked with sediment and on 62 or 17.2 per cent of the plates inoculated by the standard dilution method.

Preliminary centrifuging of a 10 cc. portion of the sample and the use of a four mm. loopful of the resulting sediment in streaking one-half of the surface of a blood agar plate was more effective in detecting the presence

TABLE 1

Detection of Streptococci with the Blood Agar Plate

METHOD OF INOCULATION	WEEKLY HEMOLYTIC STREPTOCOCCUS-LIKE COLONIES					
	Streptococci		Diphtheroids		None	
	number	*per cent*	*number*	*per cent*	*number*	*per cent*
Whole milk streaked over surface	51	14.2	0	0.0	309	85.8
Sediment streaked over surface	66	18.3	1	0.3	293	81.4
Dilution*	62	17.2	0	0.0	298	82.8

* 1 cc. of a 1 to 10 dilution plated in blood agar.

of streptococci than either of the other two methods. For this reason and because the time involved in centrifuging the sample is offset by the saving in materials and glassware, the sediment-streak method of inoculating blood agar plates is considered preferable to the other methods employed in the routine detection of streptococci by means of the blood agar plate. However, if contamination occurs at the time the samples are collected or if the samples are not placed at refrigerator temperature immediately following collection the presence of colonies of udder streptococci is more readily obscured by contaminating organisms on the sediment-streak plates than in the plates inoculated by the dilution method.

TABLE 2

Detection of Streptococci by Microscopic Examination

MATERIAL EXAMINED	STREPTOCOCCI (CHAINS OF 10 OR MORE CELLS)		COCCI OCCURRING IN PAIRS OR CHAINS OF LESS THAN 10 CELLS		NEITHER PAIRS NOR CHAINS OF COCCI	
	number	*per cent*	*number*	*per cent*	*number*	*per cent*
Stained films prepared from fresh samples	0	0.0	3	0.8	357	99.2
Stained films prepared from sediment	0	0.5	5	1.4	353	98.1
Stained films prepared from incubated milk	116	32.2	3	0.8	241	67.0

Microscopic Examination.—The results obtained by direct microscopic examination of films prepared from the fresh samples, films prepared from the sediment obtained from a centrifuged 10 cc. portion of the sample, and films prepared from the incubated sample are recorded in Table 2.

Microscopic examination of the 360 samples before incubation failed to show the presence of chains of streptococci in a single instance. Cocci occurring in pairs were observed in films prepared from three samples or only in 0.8 per cent of the total number of samples examined. Culture tests made

on these three samples yielded relatively large numbers of chain-forming streptococci, which were identified as the type most commonly responsible for chronic mastitis. Observations on many samples in addition to those included in this investigation show that chains of streptococci are rarely seen in films prepared from samples freshly drawn from udders affected with persistent chronic mastitis. However, streptococci were commonly seen in films prepared from samples collected from udders affected with an acute attack of mastitis. Although several of the 360 samples used in this investigation were abnormal in appearance, none of the animals from which the samples were obtained showed evidence of acute mastitis.

Microscopic examination of the sediment was but slightly more effective in detecting streptococci than examination of the films prepared from fresh samples. Streptococci occurring in chains were seen in the sediment from two samples, or in 0.5 per cent, and cocci occurring in pairs were seen in five instances or 1.4 per cent of the milk samples examined.

Microscopic examination of the incubated samples revealed the presence of streptococci in a surprisingly large number of the samples. Streptococci occurring in chains were found in 116 or 32.2 per cent of the 360 samples examined. Because streptococci were observed in a much smaller number of samples by the use of blood agar plates, it seemed probable that many of the streptococci seen in the incubated samples were saprophytes.

RELATION OF THE FINDING OF STREPTOCOCCI BY THE METHODS EMPLOYED TO LABORATORY EVIDENCE OF MASTITIS

In order to determine the significance of the finding of streptococci by the six methods used, the results given by each method were compared with the presence or absence of other laboratory evidence of mastitis 30 days before, at the time, and 30 days after the tests were made for the presence of streptococci. The results obtained are recorded in Table 3.

With few exceptions, the finding of streptococci by either of the three blood agar plate methods was accompanied by other evidence of mastitis. The few samples in which streptococci were detected by direct microscopic examination or by examination of films made from the sediment also gave other evidence of mastitis. The results obtained by examination of the incubated samples were of particular interest, as this method detected streptococci in a greater number of samples which showed other evidence of mastitis than did the other methods employed. Seventy-eight instances of streptococcal mastitis were detected by the miscropic examination of incubated milk samples, as compared with 61 by streaking the surface of blood agar with sediment. However, of the 116 samples in which streptococci were found following incubation, 38 failed to show other evidence of mastitis.

In general, it appears that the more sensitive the method employed for the detection of streptococci, the greater is the proportion of samples in which

TABLE 3
Relation of the Finding of Streptococci by the Methods Employed to Other Laboratory Evidence of Mastitis

METHOD	SAMPLES SHOWING STREPTOCOCCI	LABORATORY EVIDENCE OF MASTITIS DUE TO STREPTOCOCCI		
		30 days before time of test	At time of test	30 days after time of test
Blood agar plate (whole milk)	51*	45	48	47
Blood agar plate (sediment)	66*	54	61	58
Blood agar plate (dilution)	62*	53	60	58
Direct microscopic examination	3	3	3	3
Microscopic examination of sediment	7	5	7	6
Microscopic examination of incubated milk	116	62	78†	76

* Colonies resembling those of streptococci responsible for chronic bovine mastitis seen on blood agar plates.

† A total of 79 samples showed evidence of streptococci mastitis. Incubated milk failed to detect the presence of streptococci in one instance, only.

streptococci are found without other evidence of mastitis. Determination of the characteristics of strains of streptococci isolated from incubated milk obtained from quarters showing no other evidence of mastitis at the time of, or shortly after, the culture was obtained, shows that such strains are frequently saprophytes, according to Plastridge, Anderson, Brigham and Spaulding (9).

EVIDENCE OF MASTITIS IN QUARTERS YIELDING STREPTOCOCCI IN INCUBATED MILK ONLY

As shown in Table 3, 116 of the 360 samples examined contained streptococci as revealed by microscopic examination of incubated milk, while only 66 yielded colonies resembling streptococci on blood agar (see Table 1); in other words, 50 samples yielding streptococci by microscopic examination of incubated milk failed to yield visible colonies of streptococci on the blood agar plates. In order to gain information on the significance of the presence of streptococci in these samples, other evidence of mastitis at the time of the test was obtained; and the results of other tests made on the quarters invloved before and after examination of the fifty samples were considered. The results are summarized in Table 4.

Thirty of the 50 quarters involved showed no evidence of mastitis before, at the time of, or after the finding of streptococci in the incubated samples. Cultures were obtained from these samples in several instances and were

TABLE 4

Evidence of Mastitis in Quarters Yielding Streptococci in Incubated Milk Only

NUMBER OF QUARTERS	STREPTOCOCCI IN INCUBATED MILK	STREPTOCOCCI DETECTED BY BLOOD AGAR PLATES	LABORATORY EVIDENCE OF MASTITIS		
			Two weeks preceding test	At time of test	One month following test
30	+	0	0	0	0
3	+	0	0	0	+
7	+	0	0	+	+
1	+	0	0	+	0
9	+	0	+	+	+

identified as saprophytes. As the quarters from which these samples were obtained failed to shed streptococci on subsequent tests, it seems probable that the streptococci found in the incubated samples originated from outside the udder in spite of the precautions taken against contamination in collecting the samples.

On the other hand, three of the 50 quarters developed other evidence of streptococcal mastitis during the month following the finding of streptococci in the incubated samples. Cultures isolated from these quarters have been identified as the type commonly responsible for infectious chronic mastitis.

Seven quarters which gave no evidence of mastitis two weeks previously gave other evidence of mastitis at the time of the test and also on subsequent tests.

One quarter, only, gave other evidence of mastitis at the time of the test, without showing evidence of mastitis on subsequent tests.

Nine quarters known to have been affected with chronic streptococcal mastitis for some time gave samples in which the causative organism appeared in the incubated milk, but failed to do so on the blood agar plates. Blood agar plates inoculated with portions of samples collected two weeks previously and one month later revealed the presence of streptococci.

While incubated milk occasionally contains streptococci which are not responsible for mastitis, the results presented show that the presence of streptococci in the udder which cause mastitis is detected sooner by the use of incubated samples than by the inoculation of blood agar plates with portions of fresh samples, and that chronic shedders of streptococci are detected more frequently by the use of incubated milk than by the other methods used.

RELATIVE EFFICACY OF METHODS OF DETECTING STREPTOCOCCI ASSOCIATED WITH MASTITIS

Of the 360 samples included in this investigation 79 showed positive evidence of mastitis and contained streptococci, as revealed by one or several

of the tests employed; whereas 281 samples failed to show evidence at the time of making the comparisons. In order to determine the relative efficacy of the different methods used in detecting streptococci associated with mastitis, the per cent of the 79 samples yielding streptococci by each method was calculated. The incidence of the appearance on blood agar of colonies of saprophytic streptococci, diplococci or diphtheroids which resembled colonies of streptococci responsible for chronic mastitis is recorded in Table 5 under the heading "false positives." The incidence of streptococci in incubated samples from apparently healthy quarters was determined also. The incidence of false positives was calculated by dividing the number of samples in which the results of a given method indicated falsely the presence of pathogenic streptococci, by the number of samples which showed no other evidence of streptococcal mastitis.

TABLE 5

Relative Efficacy of Methods of Detecting Streptococci Associated with Mastitis

METHODS EMPLOYED	PER CENT OF TESTS WHICH GAVE	
	Positive results	False positives
Blood agar plate streaked with whole milk	60.8	1.1
Blood agar plate streaked with sediment	77.2	1.8
Blood agar plate—dilution method	76.0	0.7
Direct microscopic examination	3.8	0.0
Microscopic examination of sediment	8.9	0.0
Microscopic examination of incubated milk	98.7	13.5

The efficacy of the three methods used in inoculating blood agar plates was as follows: plates streaked with a four mm. loopful of whole milk, 60.8 per cent; plates streaked with a four mm. loopful of sediment, 77.2 per cent; and plates inoculated by the dilution method, 76.0 per cent. While the use of sediment gave a higher percentage of positive findings than the dilution method, plates inoculated with sediment gave a higher percentage of false positives, because of the higher incidence of weakly hemolytic colonies of saprophytic streptococci, diplococci or diphtheroids on plates inoculated with sediment.

On the basis of the results obtained, the finding of streptococci in films prepared from freshly drawn samples or from sediment obtained from a 10 cc. portion of the sample invariably indicated that the sample was obtained from an animal affected with streptococcal mastitis. However, these two methods detected less than 10 per cent of the 79 diseased quarters.

Microscopic examination of incubated milk was 98.7 per cent effective in detecting streptococci in samples collected from quarters affected with streptococcal mastitis. However, streptococci were observed in 38 or 13.5 per cent of the 281 samples collected from quarters which gave no other evidence of mastitis at the time. Three of the 38 quarters developed mastitis during the following month while 35 failed to do so.

DISCUSSION

In comparing the relative efficacy of six different methods used for the detection of the causative organism of chronic streptococcal bovine mastitis, 360 individual quarter samples were examined. Seventy-nine or 21.9 per cent of the samples showed laboratory evidence of mastitis and the presence of streptococci by one or more of the tests used. The incidence of positive findings given by the six methods, when used in the examination of the 79 samples collected from affected quarters was as follows:

Method used	*Per cent positive*
1. Direct microscopic examination of films (whole milk)	3.8
2. Direct microscopic examination of films (sediment)	8.9
3. Blood agar plates streaked with a 4 mm. loopful of whole milk	60.8
4. Blood agar plates inoculated by the standard dilution method (1 cc. of a 1 to 10 dilution)	76.0
5. Blood agar plates streaked with a 4 mm. loopful of sediment from 10 cc. of centrifuged milk	77.2
6. Microscopic examination of films prepared from samples incubated over-night	98.7

The samples in which streptococci were detected by direct microscopic examination of films prepared from whole milk and from sediment were from quarters that had been affected with chronic mastitis for a period of at least a year.

The per cent of the 281 samples from apparently healthy quarters, which gave weakly hemolytic colonies on blood agar that were apparently either saprophytic streptococci, diplococci or diphtheroids was as follows: Blood agar streaked with whole milk, 1.1 per cent, blood agar inoculated by the dilution method, 0.7 per cent, and blood agar streaked with sediment, 1.8 per cent.

Streptococci were detected in films prepared from incubated portions of 38 or 13.5 per cent of the 281 samples which gave no evidence at the time of the test. Three of the 38 quarters yielding these samples gave laboratory evidence of mastitis a month after the test.

CONCLUSIONS

Microscopic examination of films prepared from incubated milk revealed the presence of the causative organism of chronic streptococcal bovine mas-

titis in a larger number of instances than (1) direct microscopic examination of films prepared from whole milk or sediment, and (2) blood agar plates inoculated with a 4 mm. loopful of whole milk, or a 4 mm. loopful of sediment, or 1 cc. of a 1 to 10 dilution of the sample.

In the absence of other laboratory evidence of mastitis, the finding of streptococci in incubated samples should not be taken as conclusive evidence of infection with the organism which is commonly responsible for chronic infectious bovine mastitis. The significance of the finding of streptococci in such instances can be determined only by isolation and identification of the streptococcus found in the sample.

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LESPEDEZA HAY FOR DAIRY CATTLE

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Lespedeza is assuming a rôle of importance in the southern and south-central portions of Illinois, although it has been grown extensively in the southeastern states for a number of years. The crop has a number of uses, namely, for pasture, for seed, for hay, and for soil improvement.

Since lespedeza is a leguminous plant having a protein content similar to that of alfalfa and soybeans and can be grown on soils relatively low in productivity, it gives promise of being a means not only for improvement in the feeding of dairy cattle, but for soil improvement as well.

It was the object of the feeding trial reported herewith to measure the feeding value of lespedeza hay for milk production and for gain in weight of dairy heifers by comparing it with alfalfa hay.

Several determinations of the composition or feeding value of Lespedeza hay have been made. It is assumed that unless otherwise stated in the report that the variety was either *Lespedeza striata* (Japan clover) or *Lespedeza stipulacea* (Korean lespedeza).

Studies of *Lespedeza striata* (Japan clover) hay at the Louisiana Station (1), (2) showed a total protein content of 11.7 per cent, and a digestible protein content of 7.6 per cent. At the early bloom stage, the hay consisted of 55 per cent leaves containing 20 per cent protein and 45 per cent stems containing 8 per cent protein. Exposure of the hay to rain for several days lowered the palatability but changed the chemical composition very little.

Studies of the Korean variety (3), (5) showed that it was lower in protein than alfalfa but (3) about the same as alfalfa in its content of calcium and phosphorus. Five tests in Ohio (9) showed the lespedeza hay to contain 11.4 per cent protein. Two trials (4), (8) with dairy cows indicated that lespedeza hay was slightly less valuable than alfalfa hay while another (7) showed but little difference between alfalfa, lespedeza and soybean hays. In one of these trials (8) the cows yielded less milk but gained more weight during lespedeza hay feeding than when soybean hay was fed. Lespedeza hay fed to steers (11) gave greater gains and greater economy than alfalfa hay or soybean hay.

A study (6) of the composition of *Lespedeza sericea* hay showed it consisted of 52 per cent leaves and fine stems and contained 8.6 per cent protein. Another study (10) of hay of the same variety showed that hay harvested June 4 and 17, July 1 and 15, had protein contents of 12.3 per cent, 11.2 per cent, 10.6 per cent, and 9.5 per cent, respectively. In Ohio (9) sericea hay harvested June 28 to July 14 contained 12 to 13 per cent protein, while

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second cutting hay harvested September 20 from the same plots contained 14.5 to 17.6 per cent protein.

DESCRIPTION OF FEEDS USED IN TRIAL

The *lespedeza hay* was of the Korean variety and was grown in Sullivan County, Indiana, a few miles from the Indiana-Illinois state line. The crop was a volunteer stand and very heavy. The field was clipped and raked in July with the result that the hay crop which was harvested in September was nearly free from foreign matter. The crop was cut in early bloom. Part of the hay was cured and baled without exposure to rain. Both the leaves and stems of this part of the hay had a high green color. The remainder of the hay was exposed to rain and both leaves and stems were bleached. Both lots of hay retained the leaves on the stems almost completely. The best hay was fed to cows and the bleached hay to heifers.

The *alfalfa hay* was grown within a few miles of Urbana. That fed to the cows consisted of second cutting hay which was fine stemmed, leafy and had a high green color. The alfalfa hay fed to the heifers was second and third cutting hay somewhat coarser in quality than the other lot, but had a bright green color.

Samples of each of the four lots of hay were sent to the Bureau of Agricultural Economics of the U. S. Department of Agriculture, for grading. The grades assigned were as follows:

Kind of Hay	*Grade*	*Hay fed to*
Lespedeza (A)	U. S. No. 1 Extra Leafy Extra Green Lespedeza with 60 per cent leafiness and a trace of foreign material	Cows
Lespedeza (B)	U. S. No. 1 Extra Leafy Lespedeza with 55 per cent leafiness and 5 per cent foreign material	Heifers
Alfalfa (A)	U. S. No. 1 Extra Green Alfalfa with 80 per cent color and 45 per cent leafiness and a trace of foreign material	Cows
Alfalfa (B)	U. S. No. 1 Alfalfa with 65 per cent color, 40 per cent leafiness and a trace of foreign material	Heifers

The *corn silage* was of good quality. It was made from heavily-eared Reid Yellow Dent corn, well advanced in maturity. It contained about 32 per cent dry matter.

The *grain mixture* was made up according to the following formula: shelled corn, ground, 300 pounds; oats, ground, 320 pounds; wheat bran, 250 pounds; soybean oil meal, 100 pounds; salt, 15 pounds; steamed bone meal, 15 pounds. It contained 88.25 per cent dry matter and approximately 14 per cent total protein.

Dried beet pulp was fed to some of the highest producing cows at the rate of 4.5 pounds per head daily.

GENERAL PLAN OF FEEDING TRIAL

The trial consisted of two periods, each four weeks in length. Period I was preceded by a 1-week preliminary period and Period II by a 1-week transition period.

Two groups of cows and two groups of heifers were used. Each group of cows consisted of 8 Holsteins and 1 Jersey while the heifer groups contained animals of four breeds. The cow groups were made up by choosing two cows as nearly alike as possible with respect to breed, stage of lactation, milk yield, stage of gestation, etc., and placing one cow of this pair in Group I and the other in Group II. The heifer groups were made up in the same way except that breed, age, size, stage of gestation and amount of feed consumed daily were the factors upon which pairs were selected and division into groups was made.

The animals of a pair were fed exactly the same kind and amounts of feed except for the kind of hay. An attempt was made to have the animals consume very large amounts of hay and to this end silage was fed in small amounts to the cows while the heifers were limited to hay and grain mixture only. The refused portions of the hay were collected from the mangers frequently and placed in gunny sacks. These portions, which absorbed moisture as a result of being nosed over by the cows, were dried and the amounts calculated to the same moisture basis as the hay fed. Samples of all feeds for analysis were taken weekly.

At the close of Period 1, the groups of animals which had been fed lespedeza hay were fed alfalfa hay and the groups which had received alfalfa hay were given lespedeza hay.

The animals were weighed on three consecutive days at the beginning and end of each period and also once weekly. Each milking of each cow was weighed and sampled. Composite samples were tested weekly for butterfat content.

COMPARATIVE FEEDING VALUES

Palatability.—The lespedeza hay was less palatable than the alfalfa hay as determined by a preliminary test that was made to select animals which would consume lespedeza hay in large enough amounts for the trial. Some cows and heifers would not consume lespedeza hay as readily or in as large amounts as alfalfa hay. The fact that alfalfa hay had been fed regularly to the herd prior to this test must be considered, however.

The animals employed in the feeding trial consumed hay in large amounts per head daily and also in relation to body weight, with a negligible amount of hay refused. (Table I.)

Value for Milk Production.—The milk yields of the cows during the periods of lespedeza hay feeding were nearly the same as when alfalfa hay was fed. The actual yields were 47.04 pounds daily per cow, while lespedeza

TABLE 1
Amounts of Hay Consumed

KIND OF HAY	KIND OF ANIMALS	AMOUNT FED DAILY PER HEAD	AMOUNT REFUSED DAILY PER HEAD		AMOUNT CONSUMED DAILY	
					Per head	Per 100 lbs. live weight
		lbs.	*lbs.*	*per ct.*	*lbs.*	*lbs.*
Lespedeza (A)	Cows	21.65	.15	.69	21.50	1.66
Alfalfa (A)	"	21.65	.13	.60	21.52	1.66
Lespedeza (B)	Heifers	12.84	.06	.47	12.78	1.72
Alfalfa (B)	"	12.85	.01	.08	12.84	1.72

hay was fed, and 46.55 pounds during the alfalfa hay periods. Because of slight differences in test, a more accurate comparison is obtained by converting the milk yields to an energy basis, as shown in Table 2. While the milk yield and gain in weight were slightly greater during lespedeza hay feeding, these differences are not considered significant.

TABLE 2
Summary of Results of Feeding Lespedeza Hay in Comparison with Alfalfa Hay to Dairy Cows

KIND OF HAY	NO. OF COWS	FEED CONSUMED DAILY PER COW			GAIN IN WEIGHT DAILY PER COW	TEST OF MILK	MILK YIELD DAILY PER COW[1]
		Silage	Hay	Grain			
		lbs.	*lbs.*	*lbs.*	*lbs.*	*per cent*	*lbs.*
Lespedeza	18	19.3	21.5	14.9[2]	.6	3.81	45.7
Alfalfa	18	19.3	21.5	14.9[2]	.6	3.68	44.3

[1] Milk energy in terms of 4% milk computed according to the formula .4 × milk (in pounds) + 15 × fat (in pounds).

[2] Includes 2.2 pounds dried beet pulp.

Value for Gain in Weight.—Slightly different grades of hay than those fed to the cows were employed in the feeding trial with growing dairy heifers. The gain in weight was about 2 per cent greater during the periods of lespedeza hay feeding than during the feeding of alfalfa hay, but this difference is considered too small to be significant. The gain of approximately 1.3 pounds per head daily is satisfactory for heifers of this weight. No doubt, more rapid gains could have been obtained by reducing the amounts of hay and increasing the amounts of grain fed, but it was considered desirable for the purposes of this trial to have the hay form as large a proportion of the ration as possible.

Conditioning Effect.—Careful observations of the laxative properties of the feeds indicated that the rations containing lespedeza hay were no more or no less laxative in their effect than the alfalfa hay rations. No tests to

TABLE 3
Summary of Results of Feeding Lespedeza Hay in Comparison with Alfalfa Hay to Dairy Heifers

KIND OF HAY	NO. OF HEIFERS	LIVE WEIGHT PER HEAD	FEED CONSUMED DAILY PER HEAD		GAIN IN WEIGHT DAILY PER HEAD
			Hay	Grain	
		lbs.	*lbs.*	*lbs.*	*lbs.*
Lespedeza	23	745	12.78	3.61	1.32
Alfalfa	23	748	12.84	3.60	1.29

TABLE 4
Percentage Composition of Hays Fed

KIND OF HAY	DRY MATTER	PROTEIN	ASH	FIBER	ETHER EXTRACT	N-FREE EXTRACT	CaO	P_2O_5	LEAVES[1]
Lespedeza (A)	84.3	12.7	4.1	19.0	1.9	46.6	3.0	.4	62
Lespedeza (B)	85.9	12.8	4.1	19.1	1.7	48.2	2.8	.4	56
Alfalfa (A)	87.2	16.1	6.6	21.4	2.1	41.0	4.9	.3	49
Alfalfa (B)	88.1	16.3	6.4	26.0	2.3	37.1	4.4	.4	49

[1] Determined by separating large samples into stem and leaf portions.

determine this point were conducted, however. No harmful or undesirable effects upon the health or condition of the cattle which could be traced to the lespedeza hay feeding were observed.

Chemical Composition.—The lespedeza hay was found to be lower than the alfalfa hay in its content of protein, fiber, total ash, and lime. Judged by chemical composition alone, the lespedeza hay was inferior in feeding value to the alfalfa hay, although the lower fiber content of the lespedeza hay is a point in its favor. Evidently the hay and grain mixtures of both the lespedeza and alfalfa rations supplied plenty of protein, so that the lower protein content of the lespedeza hay was not a factor which affected the results of the feeding trial.

SUMMARY

The feeding value of lespedeza hay was compared with that of alfalfa hay in feeding trials with dairy cows and dairy heifers.

The lespedeza hay was somewhat less palatable than the alfalfa hay.

The particular lot of lespedeza hay fed to dairy cows proved practically equal pound for pound to the alfalfa hay fed, as judged by milk yields and gain in weight of the cows. Slightly lower grades of both lespedeza hay and alfalfa hay were found practically equal pound for pound for gain in weight

of growing dairy heifers. In both trials, however, it is likely that adequate amounts of protein were supplied, so that the lower protein content of the lespedeza hay was not a limiting factor.

No differences in the laxative properties or conditioning effects of lespedeza hay and alfalfa hay were observed.

The lespedeza hay was found to be lower than the alfalfa hay in its content of protein, fiber, total ash, and lime.

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THE PREPARATION OF MOLD POWDER FOR BLUE-VEINED CHEESES*

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In the manufacture of blue-veined cheeses the usual method of inoculation has been to sprinkle spores of the desired mold, in the form of a powder, over the curd at the time it is being placed in the hoops. A mold powder is commonly prepared by growing the mold on some medium and then drying and powdering the mass of material. Various methods have been suggested for growing the mold, including (a) the inoculation of baked products specially prepared from rye, whole wheat or barley flour or of the interior of a loaf of ordinary bread (2, 3, 4), and (b) dipping strips of bread into an acid suspension of mold spores (1, 5). These procedures are carried out as aseptically as possible, and the inoculated material is usually incubated at a relatively low temperature. One method of limiting the growth of foreign molds has been to gradually accustom the desired strain of mold to formalin and then dip the bread into an acid suspension of the mold spores containing 1 per cent formalin (5).

In various attempts, at the Iowa Agricultural Experiment Station, to employ the commonly suggested methods for the preparation of mold powder, practical difficulties were encountered which made advisable the use of a method which could be more satisfactorily controlled. These difficulties included (a) the development of foreign molds in the inoculated material, (b) a relatively low yield of powder because of the failure of the mold to grow in a portion of the inoculated material, and (c) the long time required for spores to develop.

METHOD SUGGESTED

After employing various procedures for preparing mold powder, the following method has been adopted for the preparation of mold powder to be used in the manufacture of Iowa Blue Cheese, a Roquefort type cheese made from cows' milk.

Medium used. Whole wheat bread is cut into cubes approximately 0.5 inch on a side and placed in salt-mouth bottles, the bottles being filled from about one-third to about one-half full. The bottles are plugged with cotton and sterilized at about 15 pounds pressure for about 30 minutes, care being taken to raise the temperature very slowly to prevent cracking of the glass. After partial cooling, the bottles are removed from the autoclave and frequently shaken to keep the pieces of bread from adhering in large masses.

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FIG. 1. TYPE OF CONTAINER USED FOR GROWING MOLD.
Left, uninoculated; right, mold well developed.

There seems to be an advantage in sprinkling the unsterilized bread with a small amount of water so that the sterilized material will not be too dry. Sterile water can also be added to the sterilized bread.

Inoculation. Two methods of inoculating the sterilized bread have been employed. The first consists of making a suspension of spores in water and adding this to the sterile material. The second and preferred method consists of sterilizing several cubes of bread in a large test tube and inoculating them with the desired mold; after sporulation has occurred the cubes are transferred to the bottles of bread and the bottles thoroughly shaken to distribute the spores. With this procedure a very heavy inoculation of the bread is easily secured.

Incubation. The inoculated bottles are incubated at 21° C. until copious spore formation has occurred. The time required varies from 8 to 12 days, depending on the strain of mold being used and, presumably, other factors.

During the incubation the bottles are shaken occasionally to insure heavy inoculation of all pieces of the bread and to keep the pieces from packing together and thus limiting the air supply. Even with frequent shaking, however, the pieces of bread overgrown with mold tend to settle together.

Drying. The bread, covered with spores, is removed from the containers and dried in a thin layer on cheesecloth supported by a frame. If desired, cheesecloth can be spread over the layer of bread. The drying is carried out in a warm room and requires from 2 to 4 days. When only a small amount of bread is being dried it can be suspended in a cheesecloth bag.

Grinding. After the material is completely dry it is pulverized in a small stone churn to which small stones have been added to serve as balls. Small amounts of bread can be ground in a mortar. The powdered material is passed through a 40-mesh screen to remove the larger particles.

Storage. The finished powder is placed in tin cans and held in a cool place until used.

GENERAL RESULTS WITH MODIFICATION OF THE METHOD

Various modifications of the method, as outlined, have been used. White bread did not seem to support as good a growth of the molds employed as whole wheat bread. Cracker crumbs were less satisfactory than white bread, probably because the crackers tended to pack into large masses which prevented easy access of air. The addition of an aqueous solution of lactic acid did not appear to improve the growth of the molds with any of the materials used. Large Erlenmeyer or round flasks were employed but seemed to be less satisfactory than the salt-mouth bottles. The larger openings in the bottles may provide a better air supply than the smaller openings in the flasks.

SPORE CONTENTS OF POWDERS

The spore contents of the powders that have been made varied from 30 million to 1.5 billion per gram, as determined by the plate method using tomato agar as the medium, with most of the powders having spore contents between 100 million and 300 million per gram. When the spores were suspended in water and a count made with a haemocytometer, much higher values were secured; with this procedure many of the spores were seen to be in groups.

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A METHOD FOR THE MICROSCOPIC EXAMINATION OF BUTTER

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Many of the organisms of importance in the sanitary and industrial control of butter are not easily detected by routine plating procedures, therefore microscopic examination of butter is advantageous under many circumstances. Hammer and Nelson (1) devised a method which involves the separation of the fatty and non-fatty portions of the butter with the aid of heat and centrifugal force; 0.01 cc. of the non-fatty portion is spread over 1, 4, or 8 square centimeters, stained, and examined by the technique described by Breed (2). The value of such a method for both research and control purposes is obvious.

The following method was designed primarily to eliminate some of the practical difficulties involved in the use of the 0.01 cc. capillary pipette and the centrifuge, and to provide a method which would lend itself to routine examination of large numbers of samples.

The butter to be examined is melted by heating to 45° C. for a period not to exceed 15 minutes, after which it is agitated sufficiently to insure a homogeneous sample.

(a) On a standard 1×3 inch, chemically clean, glass slide place 0.1 cc. of the melted butter, 1 drop of xylol, and 1 drop of Mayer's egg-glycerine mixture. To prepare Mayer's solution equal parts of egg albumin and glycerine are mixed, thoroughly beaten, and filtered through cotton. One per cent sodium salicylate should be added as a preservative, and the solution kept in a refrigerator when not in use. Mayer's solution is used to aid in fixing the smear to the slide.

(b) With an "L" shaped platinum needle stir the butter, Mayer's solution, and xylol together until the mixture is opalescent and homogeneous, then spread evenly over the entire area of the slide. It is important that the mixing of the ingredients be properly done before spreading the smear over the area of the slide. Proper mixing requires from 2 to 3 minutes.

(c) Allow the preparation to dry, and at the same time coagulate the albumin by placing the slide on a flat bottle of hot water at approximately 80° C. for 10 to 15 minutes.

(d) Fix the smear in 70 per cent alcohol for 10 to 20 seconds, and allow to dry.

(e) Immerse in xylol 2 minutes, and allow to dry.

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(f) Stain in methylene blue for 1 minute, then wash by dipping the slide once in each of two vessels of water. Allow to dry in the air.

(g) Examine under an oil immersion lens, the field of which has been standardized to a diameter of 0.157 mm. (3). The average number of microorganisms per field multiplied by 1,000,000 gives the number per cubic centimeter of melted butter.

If it is desirable to enumerate objects large enough to be seen with the lower magnifications of the microscope, standardize the diameter of the field of the high dry objective to 0.351 mm. and of the low power objective to 1.57 mm.; multiply the average number of objects observed per field by the factors 200,000 and 10,000 respectively to give the number per cubic centimeter of melted butter. The method described in this paper has been found to be equally advantageous for the microscopic examination of heavy cream.

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VITAMIN D STUDIES IN CATTLE

II. THE VITAMIN D SPARING ACTION OF MAGNESIUM IN THE RATION OF DAIRY CATTLE*

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The possibility of a vitamin D sparing effect of magnesium was noted by Huffman and coworkers (1) in 1930, when Calf C–44 recovered from rickets after commercial magnesium carbonate had been added to the ration. When this supplement was withdrawn from the ration, however, the rachitic symptoms again became pronounced. Several other investigators have made similar observations. von Euler and Rydbom (2) working with rats reported that the addition of magnesium salts to a rachitogenic diet had an antirachitic effect. They explained the results which they obtained upon the basis of increased phosphatase activity due to the magnesium ions. Kay (3) has also demonstrated that magnesium ions stimulate phosphatase activity. Barbour and Winter (4) found a definite calcium retention in dogs which had been fed a basal diet supplemented with magnesium in the form of the gluconate or the lactate. Carswell and Winter (5) also found that magnesium appeared to favor calcium storage in man when the phosphorus intake was adequate. Becka (6) reported that certain magnesium compounds prevented or cured osteomalacia in cattle. Some investigators (7) (8), however, have reported that the addition of magnesium compounds to the diets have resulted in the disturbance of the calcium and phosphorus metabolism. The earlier work on the problem has been reviewed by Elmslie and Steenbock (9).

In a previous report (10), in which the antirachitic value of sun-cured alfalfa was demonstrated, it was suggested that some portion of that value may have been due to the high magnesium content of the hay. The purpose of this investigation was to study the relationship of magnesium to rickets in calves when the basal rachitogenic ration was supplemented with (a) magnesium in the form of the carbonate or the oxide and (b) the efficacy of these compounds when a limited amount of vitamin D was introduced into the ration.

EXPERIMENTAL

Twelve healthy grade Holstein calves were used in this work. Calves C–99, C–101, C–104 and C–107 received whole milk up to 45 days of age, after which time the whole milk was replaced with skim milk, corn and oats.

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The corn and oats were replaced at about 90 days of age by a rachitogenic grain mixture, the composition of which appears below:

Yellow corn	50 per cent	Linseed oil meal	8.5 per cent
Oats	20 per cent	Calcium carbonate	1.0 per cent
Corn gluten meal	20 per cent	Sodium chloride	0.5 per cent

The skim milk was discontinued at about 120 days of age. C–99 received 10 cc. of cod liver oil per day and alfalfa hay *ad lib.* to 90 days of age in addition to the regular ration. C–159 had been used in another experiment and was not placed on the basal rachitogenic ration until 296 days of age.

Calves C–170, C–172, C–173, C–174, C–175 and C–176 were fed whole milk up to 30 days of age, after which time they received skim milk, corn and oats, and 5 cc. of cod liver oil per day. The corn and oats were replaced by the rachitogenic grain mixture at 90 days of age but the skim milk was not discontinued until 120 days of age. In addition to the above rations, C–172 and C–175 received one per cent of the dry matter of the ration as $MgCO_3$ (C.P.) and C–174 and C–176 received an equivalent amount of magnesium as MgO (U.S.P.). The actual magnesium intake of each calf was the same. Calf C–89 received the same basal ration as C–44 (1), namely, a grain mixture and wheat straw.

The calves were kept in the barn during the day but were turned out for exercise at night.

Shavings were used for bedding. The calves were offered water twice daily. Blood samples were taken from the jugular vein at least every two weeks and the plasma analyzed for calcium, inorganic phosphorus (10) and magnesium (11) by methods previously reported. The 8th rib on the right side, a section of the orbital plate and the right dental pad were used for the determination of ash, calcium, phosphorus and magnesium by methods which have already been recorded (10).

RESULTS

The results for a representative animal of each group are shown in Tables 1–5 and in Figure 1. The ash contents of the moisture-free, fat-free 8th rib, orbital plate and dental pad are summarized in Table 6.

Effect of Supplementing the Rachitogenic Ration with Magnesium Compounds

Calf C–101. This calf manifested tetany at 95 days of age and again on the 106th day, at which time the calcium and inorganic phosphorus were 6.2 and 8.01 mg. per 100 cc. of plasma. Tetany was again manifested two days later. The concentrations of calcium and inorganic phosphorus during this convulsion were 6.5 and 7.53 mg. Up to this time the calcium, phosphorus and magnesium intakes had averaged 7.0, 7.0 and 1.5 gm. per day respectively. Magnesium carbonate was added to the ration at this time which

TABLE 1

C–104. Effect of the Rachitogenic Ration, Supplemented with Magnesium Carbonate, Upon the Plasma Calcium, Inorganic Phosphorus and Magnesium

DATE	AGE	WEIGHT	PLASMA		
			Ca	P	Mg
	days	*lbs.*	*mg. per 100 cc.*		
1–28–31	90[1]	176	9.4	6.32	.....
2–7	100	182	7.6	7.58	.....
2–17	110	189	7.3	7.27	1.95
2–27	120	197	.....	.....	.....
3–7	130	207	7.7	6.98	2.10
3–17	140[2]	211	7.8	5.37	3.47
3–27	150	213	.....	.....	.....
4–8	160	211	8.2	5.17	2.83
4–18	170	221	7.7	5.93	1.56
4–28	180	230	9.1	5.06	2.66
5–8	190	239	7.4	5.63	2.86
5–12	194	244	7.7	5.14	2.13

[1] $MgCO_3$ added to ration at 91 days of age.

[2] Legs started to bow.

TABLE 2

C–89. Effect of a Magnesium Carbonate Supplement Upon the Plasma Calcium and Inorganic Phosphorus When Wheat Straw Furnished the Only Source of Vitamin D

DATE	AGE	WEIGHT	PLASMA	
			Ca	P
	days	*lbs.*	*mg. per 100 cc.*	
11–25–30	367	465	10.2	9.33
12–5	377	488	11.4	8.33
12–15	387	491	10.8	8.56
12–25	397	501	10.0	8.47
1–4–31	407	522	8.3	9.40
1–14	417	527	7.6	7.76
1–24	427	536	10.2	8.62
2–3	437	564	6.7	6.90
2–13	447	580	6.8	7.67
2–23	457	581	7.3	8.93
3–2	465[1]	down	6.3	6.96
3–15	477	"	7.9	6.20
3–25	487	"	9.8	5.78
4–4	497	"	11.0	5.39
4–14	507	"	10.4	5.67
4–21	514	"	9.5	6.28

[1] $MgCO_3$ added to ration at 464 days of age.

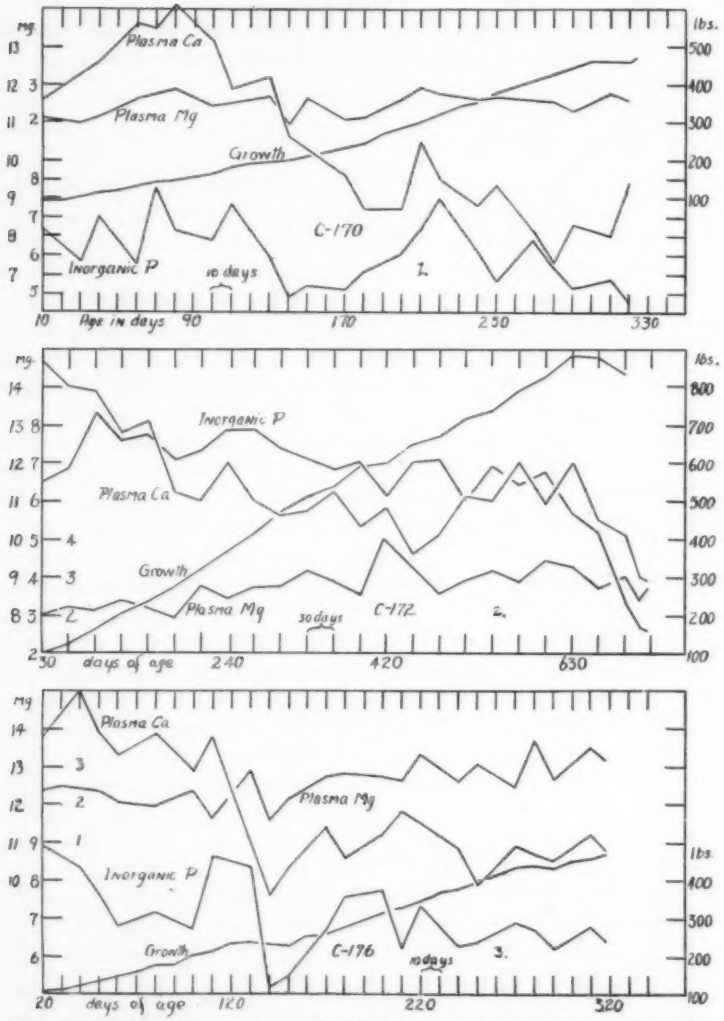


FIG. 1. Plasma calcium, inorganic phosphorus and magnesium curves of calves which received the rachitogenic ration plus 5 cc. of cod liver oil per day, with and without a magnesium supplement.

increased the magnesium intake to 10 gm. per day but the calcium intake decreased because of anorexia. Tetany was not observed again until the calf was 142 days of age. By this time the plasma inorganic phosphorus and magnesium were also much below normal for a calf of this age. The

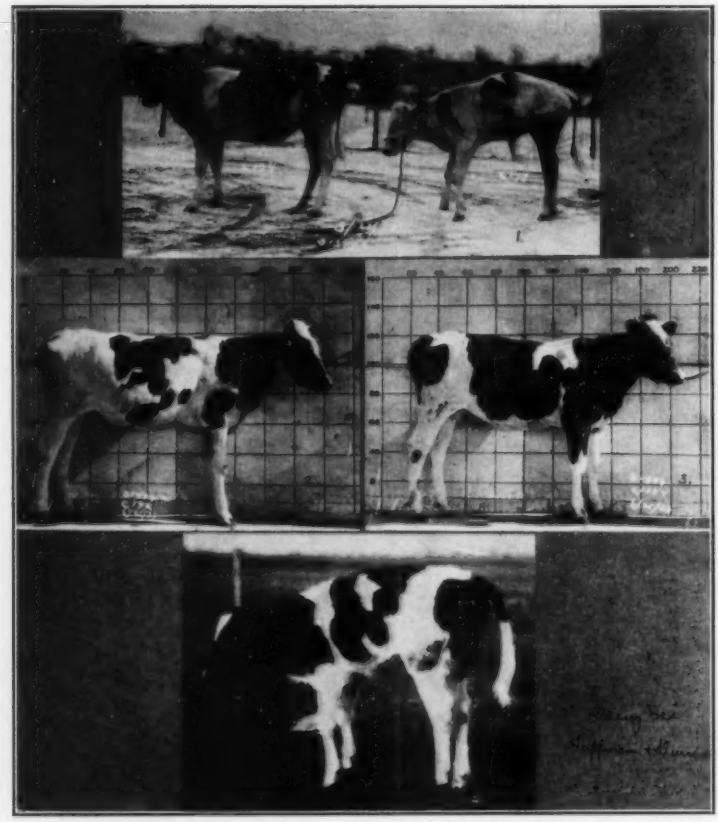
calf was unable to use its hind quarters following this attack. Two days later it was given 30 cc. of standard cod liver oil and 100 units of parathormone. Another 100 units were given on the following day in an attempt to bring about recovery from coma. On the next day the calf had recovered from the comatose state while the calcium, inorganic phosphorus and magnesium were 5.6, 4.35 and 1.01 mg. per 100 cc. respectively. Although the ration was changed to skim milk and sprouted oats at this time the inorganic phosphorus continued to decline. C–101 died at 176 days of age. A fractured femur was found at autopsy in addition to the severely rachitic bones.

Calf C–104. This calf was bled at 90 days of age, at which time the concentrations of calcium and inorganic phosphorus were 9.4 and 6.32 mg. per 100 cc. of plasma. The rachitic grain mixture containing 3 per cent magnesium carbonate replaced the corn and oats at 91 days of age. The change to the high magnesium ration did not aid in maintaining the plasma calcium and phosphorus at normal levels. The first evidence of rickets was observed at 137 days of age but the concentrations of calcium and inorganic phosphorus had already declined to 7.8 and 5.37 mg. The intakes of calcium, phosphorus and magnesium were 7.0, 7.4 and 1.5 gm. per day respectively previous to the addition of the magnesium carbonate, but after the addition, the intakes increased to 15.6, 9.8 and 11.0 gm. per day respectively. When the calf was slaughtered at 194 days of age the plasma calcium, inorganic phosphorus and magnesium values were 7.7, 5.14 and 2.13 mg. respectively. The rib bones were rachitic. Table 1 shows the typical blood findings of the calves in this group, although the inorganic phosphorus declined to much lower levels in some of the calves.

Calf C–107. When this calf was first bled at 83 days of age the calcium and inorganic phosphorus were 8.9 and 6.48 mg. per 100 cc. of plasma. The addition of three per cent of the ration as magnesium carbonate at 91 days of age did not prevent the manifestation of rickets at 155 days of age. The concentrations of calcium and inorganic phosphorus had decreased to 8.0 and 3.77 mg. by this time. When the calf was slaughtered at 200 days of age these values were 9.7 and 2.35 mg. Tetany was not observed in this calf.

Calf C–99. This calf received the basal ration supplemented with 10 cc. of cod liver oil per day and sun-cured alfalfa hay *ad lib.* to 90 days of age. The blood values were normal at 120 days of age but during the next 60 days the calcium and inorganic phosphorus had steadily dropped to 7.3 and 5.68 mg. per 100 cc. of plasma. Stiffness was not observed until the calf was 205 days of age. The rachitic condition increased in severity, consequently 3 per cent of the rachitic grain mixture was replaced with magnesium carbonate at 234 days of age. Plate I illustrates the physical condition of the calf at this time. The plasma calcium, inorganic phosphorus and magnesium values were 6.3, 5.13 and 1.47 mg. respectively. The ingestion of the magnesium salt failed to relieve the stiffness and caused the appetite to de-

PLATE I



1. C–173 received the rachitogenic ration plus 5 cc. of cod liver oil per day. Typical rachitic stance. C–174 received the same ration as C–173 supplemented with magnesium oxide.

2. C–172 received the same ration as C–174, no evidence of rickets at 410 days of age.

3. C–174. No evidence of rickets at 404 days of age.

4. C–99. Typical rachitic stance, one rear foot directly behind the other one and straight pasterns. Front legs bowed forward and outward.

crease 50 per cent, but it temporarily increased the plasma calcium to 8.1 mg. and the magnesium to 3.77 mg. There were no significant variations in the inorganic phosphorus. During the development of rickets the calcium, phosphorus and magnesium intakes were 11.5, 7.6 and 4.2 gm. respectively. Tetany was not observed in this calf. C–99 died at 256 days of age

at which time the concentrations of calcium, inorganic phosphorus and magnesium in the plasma were 6.9, 6.83 and 2.53 mg. respectively. The ribs were markedly rachitic.

Calf C–159. This calf had been used previously to determine the curative value of viosterol. At 296 days of age the viosterol was discontinued and the calf was placed on the basal rachitic ration. The plasma calcium maintained its normal position until 366 days of age when it abruptly dropped to 8.1 mg. per 100 cc. and progressively declined to a minimum of 6.1 mg. at 395 days of age. Stiffness was also observed at this time. Therefore, sufficient magnesium oxide was added to the ration to increase the magnesium intake from 6.9 to 16.0 gm. per day. In the absence of vitamin D, the addition of the magnesium oxide failed to alleviate the hypocalcemia and did not prevent the rachitic condition from becoming more severe. The calf was removed from this phase of the experiment at 436 days of age.

Effect of Supplementing the Rachitogenic Ration with a Small Amount of Vitamin D and Magnesium Compounds

Calf C–89. This calf received the same ration as C–44 (1) which consisted of a grain mixture and wheat straw. C–89 developed a case of low calcium, normal phosphorus rickets by 462 days of age. The animal was also unable to use its hind quarters after this date. As indicated in Table 2, 5 per cent of magnesium carbonate was added to the ration at 464 days of age. The concentrations of calcium and inorganic phosphorus were 5.9 and 6.25 mg. per 100 cc. of plasma on this day. The change in the ration led to an immediate and considerable increase in the plasma calcium but only caused insignificant variations in the inorganic phosphorus. When this animal was slaughtered at 514 days of age, post-mortem examination revealed a fractured vertebra which had completely calcified and bones which failed to show the gross evidence of rickets.

The addition of magnesium supplements to the rachitogenic rations of C–99, C–101, C–104, C–107 and C–159 failed to prevent or to cure rickets in the absence of vitamin D but the marked response which had been observed in C–89 indicated the possibility that the ingested magnesium might have exerted a vitamin D sparing action. We (12) had previously demonstrated that 10 cc. of standard cod liver oil per day protected heifers from rickets to about 15 months of age after which time the plasma calcium and inorganic phosphorus values became subnormal. Five cc. of standard cod liver oil per day, which furnished insufficient vitamin D to meet the requirements, were added to the basal rachitic ration of 6 calves. The calcium, phosphorus and magnesium intakes of calves C–170 and C–173, which were fed the basal ration plus 5 cc. of cod liver oil, were approximately 10.0, 7.0 and 2.0 gm. per day respectively up to 6 months of age and approximately 16.5, 11.0 and 6.5 gm. per day from 6 to 10 months of age. C–174 and C–176

were fed the same ration supplemented with magnesium oxide and C–172 and C–175 received the same ration supplemented with magnesium carbonate. The calcium and phosphorus intakes were the same as for C–170 and C–173 but the magnesium intake was 7 gm. per day to 6 months of age and 16 gm. per day from 6 to 10 months of age.

Calf C–170. The body weights, plasma calcium, inorganic phosphorus and magnesium values appear in Table 3. During the first 4 months of age

TABLE 3

C–170. Effect of the Rachitogenic Ration, Supplemented With 5 cc. of Cod Liver Oil Per Day, Upon the Plasma Calcium, Inorganic Phosphorus and Magnesium

DATE	AGE	WT.	PLASMA			DATE	AGE	WT.	PLASMA		
			Ca	P	Mg				Ca	P	Mg
	days	*lbs.*	*mg. per 100 cc.*				*days*	*lbs.*	*mg. per 100 cc.*		
5–11–32	10	97	11.6	6.69	2.12	10–28	180	243	8.7	5.58	2.13
5–21	20	96	.....	.....	.....	11–7	190	269	.....	.....	.....
5–31	30	103	.....	5.81	1.99	11–17	200	286	8.7	6.54	2.57
6–10	40	111	12.6	7.02	2.14	11–27	210	301	10.5	6.69	2.93
6–20	50	118	13.6	5.74	2.62	12–7	220	326	9.5	7.53	2.75
6–30	60	128	.....	.....	.....	12–17	230	345	.....	.....	.....
7–10	70	140	13.5	7.76	2.74	12–27	240	355	8.8	6.13	2.60
7–20	80	146	14.1	6.65	2.88	1–6–33	250	377	9.3	5.32	2.69
7–30	90	153	.....	.....	.....	1–16	260[1]	396	.....	.....	.....
8–9	100	161	13.2	6.38	2.45	1–26	270	411	8.1	6.41	2.62
8–19	110	178	11.9	7.35	2.51	2–5	280	429	7.3	5.71	2.58
8–29	120	189	.....	.....	.....	2–15	290	448	8.3	5.12	2.33
9–8	130	194	12.2	5.95	2.69	2–25	300	462	.....	.....	.....
9–18	140	198	10.6	4.90	1.90	3–7	310	463	8.0	5.34	2.78
9–28	150	208	.....	5.17	2.63	3–17	320	461	9.4	4.73	2.59
10–8	160	220	.....	.....	.....	3–23	326	473	.....	.....	.....
10–18	170	230	9.6	5.10	2.08						

[1] Stiff at 266 days of age.

this calf had a few inorganic phosphorus values which were below 6.5 mg. per 100 cc. of plasma, whereas all of the calcium values maintained a normal position throughout this period. During the fifth and sixth months of age the inorganic phosphorus values were consistently below normal. They returned to normal during the seventh month, then progressively declined to a minimum of 4.73 mg. at the end of the survival period. The calcium values did not drop to below normal until the sixth month. They increased slightly during the seventh month, after which time subnormal values prevailed to the end of the experiment. The increased concentrations of calcium and inorganic phosphorus during the seventh month were associated with an increased appetite for grain, thereby causing an appreciable increase in the calcium, phosphorus and magnesium intakes. The plasma magnesium values also increased during this period and maintained a normal position until the end of the experiment. Rickets, as indicated by stiffness, were first observed at 266 days of age. The condition rapidly became more pronounced and by the time of slaughter at 326 days of age, the calf was very stiff.

Calf C–173. This calf grew at a slower rate and evidenced stiffness at an earlier age than C–170. The plasma calcium values were normal throughout the experiment, with few exceptions. The inorganic phosphorus values, however, were all below normal after 90 days of age. Stiffness was first observed at 220 days of age when the calcium and inorganic phosphorus values were 11.3 and 6.07 mg. per 100 cc. of plasma. The inorganic phosphorus continued to drop until at slaughter, at 336 days of age, it had reached a minimum of 3.90 mg. During the time when rickets were most pronounced, the plasma magnesium values were normal. The rachitic condition of this calf is shown in Plate I.

Calf C–172. This calf received the basal ration supplemented with magnesium carbonate until 11 months of age, at which time the carbonate was replaced by magnesium oxide. This animal was continued on experiment to determine the duration of the vitamin D sparing action of the magnesium oxide. The results are shown in Table 4. The plasma calcium and inor-

TABLE 4

C–172. Vitamin D Sparing Effect of Magnesium Carbonate When Fed as a Supplement to the Rachitogenic Ration Plus 5 cc. of Cod Liver Oil Per Day

DATE	AGE	WT.	PLASMA			DATE	AGE	WT.	PLASMA		
			Ca	P	Mg				Ca	P	Mg
	days	*lbs.*	*mg. per 100 cc.*				*days*	*lbs.*	*mg. per 100 cc.*		
5–27–32	20	98	14.7	6.51	2.03	1–2–33	240	373	.....	.....	.....
6–6	30	108	.....	.....	.....	1–12	250	410	11.5	7.81	2.91
6–16	40	115	14.6	8.23	2.29	1–22	260	408	11.2	8.39	2.75
6–26	50	121	13.8	7.81	2.39	2–1	270	425	10.3	7.40	2.53
7–6	60	131	13.5	4.50	1.95	2–11	280	446	.....	.....	.....
7–16	70	145	.....	.....	.....	2–21	290	469	10.8	7.86	2.32
7–26	80	161	14.1	8.93	2.15	3–3	300	479	10.5	6.95	3.18
8–5	90	178	13.8	7.71	2.17	4–2	330[1]	508	10.7	7.10	3.15
8–15	100	184	.....	.....	.....	5–2	360	553	11.2	6.83	2.87
8–25	110	204	12.6	7.76	2.06	6–1	390	589	10.4	7.04	2.51
9–4	120	223	13.1	7.49	2.71	7–1	420	599	10.8	6.12	4.05
9–14	130	223	13.1	6.69	2.07	7–31	450	647	9.6	7.03	3.25
9–24	140	240	.....	.....	.....	8–30	480	667	10.1	7.09	2.56
10–4	150	246	.....	8.81	2.34	9–29	510	715	11.1	6.04	2.91
10–14	160	258	11.2	7.27	1.92	10–29	540	735	11.0	6.90	3.17
10–24	170	268	.....	.....	.....	11–28	570	790	12.1	6.41	2.88
11–3	180	299	11.2	6.87	1.91	12–28	600[3]	824	11.0	6.74	3.41
11–13	190	302	10.2	7.53	2.62	1–27–34	630	881	12.0	5.65	3.28
11–23	200	318	11.8	7.14	2.90	2–26	660[5]	878	10.6	5.17	2.70
12–3	210	337	.....	.....	.....	3–28	690[4]	837	10.1	3.34	3.01
12–13	220	353	11.9	8.33	2.08	4–16	709[5]	.....	9.5	2.35	2.67
12–23	230	369	12.2	7.44	2.82	4–23	716[6]	.....	8.9	2.60	2.73

[1] Changed to MgO. [2] First evidence of stiffness. [3] Stiff. [4] Hocks enlarged. [5] Calved. [6] Died.

ganic phosphorus values were normal, with very few exceptions, to 20 months of age. The normal condition of this animal is shown in Plate I. The clinical evidence of rickets coincided with the first manifestation of stiffness at 602 days of age. The calcium and inorganic phosphorus values

were 11.0 and 6.74 mg. per 100 cc. of plasma at 600 days of age. This animal developed all of the clinical symptoms of rickets yet the plasma calcium and inorganic phosphorus values remained within the normal limits. Following the first evidence of stiffness, however, the inorganic phosphorus steadily dropped. C–172 was bred at 447 days of age. The demand for additional vitamin D for gestation undoubtedly aggravated the rachitic condition. Both the calcium and inorganic phosphorus had decreased to 9.5 and 2.35 mg. at the time of calving. C–172 died 7 days following calving, at which time these values were 8.9 and 2.60 mg.

Calf C–175. This calf also received magnesium carbonate as a supplement to the rachitogenic ration. The inorganic phosphorus values were within the normal range throughout the entire experiment. The plasma calcium values were also normal with the exception of the four terminal values which were only slightly subnormal. The terminal calcium, inorganic phosphorus and magnesium values were 9.4, 8.50 and 3.31 mg. per 100 cc. of plasma respectively. This animal did not manifest stiffness at any time. It was slaughtered at 320 days of age.

Calf C–174. This calf received the basal ration supplemented with magnesium oxide and was kept on this ration to determine the duration of the effectiveness of the magnesium supplement in preventing rickets. The condition of this animal at 404 days of age is shown in Plate I. C–174 was bred at 448 days of age and conceived at this service but she aborted at 524 days of age. The anorexia, which was associated with the premature expulsion of the fetus, exaggerated the rachitic condition. Stiffness was first observed at 583 days of age. The plasma calcium values were all within the normal range during the experiment and only 6 subnormal inorganic phosphorus values were observed from birth to 500 days of age. After this age the inorganic phosphorus slowly declined to a minimum of 3.93 mg. per 100 cc. of plasma. C–174 developed normal calcium, low inorganic phosphorus rickets. She was slaughtered at 602 days of age, at which time the plasma calcium, inorganic phosphorus and magnesium values were 11.9, 5.43 and 3.08 mg. respectively.

Calf C–176. This calf received the basal ration supplemented with magnesium oxide. The results are shown in Table 5. Almost all of the plasma calcium and inorganic phosphorus values were within the normal range. There were only two calcium values below 10.0 mg. per 100 cc. (9.6 and 9.9 mg.) and only two inorganic phosphorus values were below 6.0 mg. (5.17 and 5.48 mg.).

There was no evidence of stiffness. To all outward appearances this animal was normal at the time of slaughter at 317 days of age. The terminal plasma calcium, inorganic phosphorus and magnesium values were 10.8, 6.41 and 3.18 mg. respectively.

Post-mortem examination of the long bones of the animals receiving a

TABLE 5

C–176. Vitamin D Sparing Effect of Magnesium Oxide When Fed as a Supplement to the Rachitogenic Ration Plus 5 cc. of Cod Liver Oil Per Day

DATE	AGE	WT.	PLASMA			DATE	AGE	WT.	PLASMA		
			Ca	P	Mg				Ca	P	Mg
	days	*lbs.*	*mg. per 100 cc.*				*days*	*lbs.*	*mg. per 100 cc.*		
6–18–32	20	108	13.8	8.87	2.40	11–25	180	277	10.6	7.58	2.80
6–28	30	110	15.0	8.83	2.47	12–5	190	296	.....	.....	.....
7–8	40	121	.....	.....	.....	12–15	200	311	11.2	7.71	2.74
7–18	50	131	13.9	7.62	2.36	12–25	210	328	11.8	6.22	2.62
7–28	60	142	13.3	6.79	2.07	1–4–33	220	345	11.5	7.35	3.31
8–7	70	158	.....	.....	.....	1–14	230	367	.....	.....	.....
8–17	80	176	13.9	7.14	1.98	1–24	240	373	10.8	6.28	2.60
8–27	90	177	.....	.....	.....	2–3	250	399	9.9	6.38	3.06
9–6	100	202	12.9	6.72	2.37	2–13	260	416	.....	.....	.....
9–16	110	215	13.8	8.62	1.63	2–23	270	433	10.9	6.87	2.44
9–26	120	233	.....	.....	.....	3–5	280	440	10.7	6.69	3.68
10–6	130	237	.....	8.33	2.91	3.15	290	434	10.5	6.22	2.67
10–16	140	230	9.6	5.17	1.56	3–25	300	453	.....	.....	.....
10–26	150	228	10.3	5.48	2.17	4–4	310	459	11.2	6.79	3.52
11–5	160	250	.....	.....	.....	4–11	317	470	10.8	6.41	3.18
11–15	170	262	11.4	6.69	2.74						

TABLE 6

Ash and Percentage Composition of Bones and Terminal Plasma Values of Calves which Received the Rachitogenic Ration, Supplemented with Magnesium Carbonate or the Oxide, With and Without Cod Liver Oil

CALF	BONE	AGE	WT.	ASH	Ca	P	Mg	PLASMA		
								Ca	P	Mg
No.		*days*	*lbs.*	*%*	*%*	*%*	*%*	*mg. per 100 cc.*		
C–170	8th	323	473	45.36	17.41	8.10	0.49	9.4	4.73	2.59
C–173	right	319	323	44.98	17.75	8.18	0.66	9.0	3.90	2.93
C–172	rib	718	837	49.60	19.98	9.06	0.80	8.9	2.60	2.73
C–172's		0[1]	74	47.60	18.20	8.71	0.51			
C–174		602	740	50.80	19.60	9.05	0.82	11.9	5.43	3.08
C–175		318	565	50.90	19.37	9.24	0.74	9.4	8.50	3.31
C–176		317	470	49.45	19.80	9.12	0.66	10.8	6.41	3.18
C–99	Orbital	256	255	47.61				6.9	6.83	2.53
C–104	Plate	194	244	45.77				7.7	5.14	2.13
C–170				46.80	18.71	8.35	0.57			
C–173				44.20	17.43	7.94	0.66			
C–89		514	down	50.82				9.5	6.28	
C–174				53.85	21.00	9.56	0.80			
C–175				53.35	20.91	9.52	0.91			
C–176				51.00	20.45	9.08	0.86			
C–99	Dental			48.96						
C–104	Pad			47.89						
C–107		206	154	42.51				9.7	2.35	2.47
C–170				46.86	17.35	8.27	0.58			
C–173				46.95	18.59	8.16	0.63			
C–89				54.79						
C–174				54.50	21.25	9.62	0.95			
C–175				50.25	19.50	8.77	0.72			
C–176				50.20	20.30	8.86	0.75			

[1] Full term calf, stillbirth.

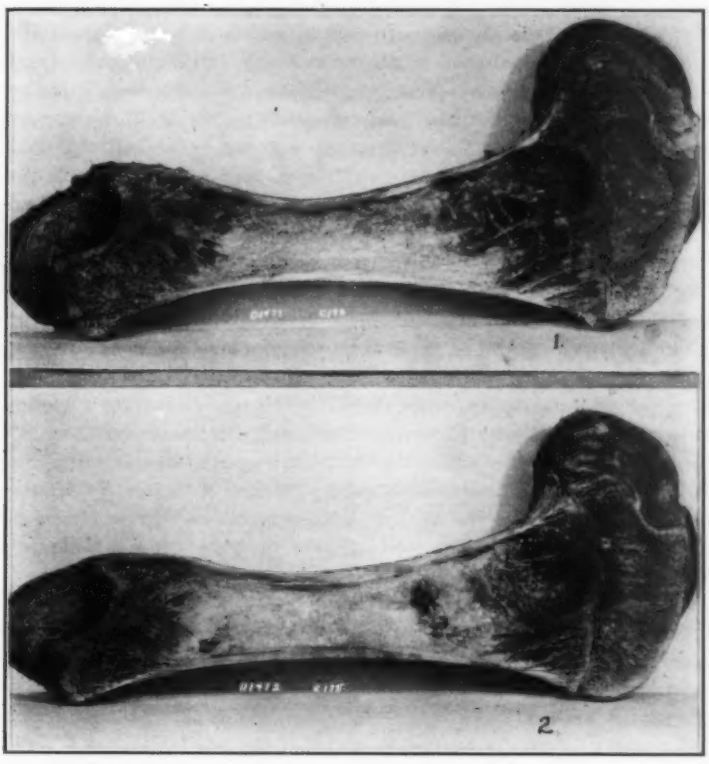
magnesium supplement showed these bones to be in almost as poor condition as similar bones from the animals which did not receive a magnesium supplement. There was a pronounced difference, however, between the two groups when the costochondral junctions were compared. In C–170 and C–173 these junctions had considerable uncalcified osteoid tissue while in C–175 and C–176 these areas were well calcified, yet upon gross examination, the ribs of the two latter animals appeared to be soft. The ash values for the 8th rib, orbital plate and dental pad were higher for the latter two animals than for the two calves which did not receive a magnesium supplement. The ash and mineral analyses are summarized in Table 6. Plate II shows the lack of trabeculation in the femur of C–170 and the improved trabeculation in the femur of C–175.

DISCUSSION

It was evident from the results with calves C–99, C–101, C–104, C–107 and C–159 that the addition of either magnesium carbonate or the oxide to the rachitogenic ration failed to produce antirachitic effects. These results are in agreement with other workers (7) (8) who reported that the addition of magnesium compounds to rachitogenic diets either failed to cure rickets or interfered with normal bone formation. The results with C–89, however, indicated that the addition of magnesium carbonate to a ration containing wheat straw aided in restoring the blood calcium to normal and also revealed at autopsy, the complete calcification of a previously fractured vertebra. Oat straw has been shown to contain some vitamin D (13). Both C–170 and C–173, which received the rachitogenic ration plus 5 cc. of cod liver oil, developed marked rickets, which were indicated by a prompt and continuous decline in the concentrations of calcium and inorganic phosphorus in the plasma and by stiffness. The addition of magnesium supplements to the rachitogenic rations of C–172 and C–175 (MgO) and C–174 and C–176 ($MgCO_3$) demonstrated that these supplements were only effective in the prevention of rickets when a small amount of vitamin D was present in the ration.

The magnesium supplements aided in maintaining the normal concentrations of calcium and inorganic phosphorus in the blood of the calves up to 10.5 months of age when 5 cc. of cod liver oil per day were added to the ration. There was also a tendency, although not marked, for the concentration of magnesium to increase in the plasma, but the concentration never increased sufficiently to produce drowsiness. Hirschfelder (14) reported that coma set in when the concentration of magnesium reached 17 mg. per 100 cc. and that a number of patients showed a decided drowsiness or even slight coma when the concentrations were only about two-thirds of this value. There is a slight possibility that the moderate increase in the plasma magnesium may have reduced the sensation of pain so that stiffness did not take

PLATE II



1. Lack of trabeculae in a femur from C–170.
2. Improved trabeculation in a femur from C–175.

place in these calves. There were no manifestations of clinical rickets by these animals up to 10.5 months of age but the rachitic conditions of C–170 and C–173 were severe when these calves were slaughtered at the above age.

The two animals, C–172 and C–174, which were kept on the rachitic ration supplemented with 5 cc. of cod liver oil per day and a magnesium supplement, did not become stiff until about 20 months of age. In the case of these young cows, whose skeletons could not have been as completely calcified as fully mature cows, the mineral drain in favor of the fetus was especially serious. The degree of calcification of C–172's calf was not appreciably modified by the apparent lack of vitamin D as the ash and mineral analyses of the 8th rib were less than 10 per cent below the normal values for a new-born calf.

Although the plasma calcium and inorganic phosphorus values and the absence of stiffness of the calves fed the magnesium supplements indicated better calcium and phosphorus utilization, yet their bones appeared soft. The long bones appeared to be in almost as poor condition as those from the calves receiving the rachitogenic ration without the magnesium supplement. Plate II illustrates the condition and amount of trabeculae in a comparative longitudinal section of a femur from each group. The ash and mineral values of the 8th rib, orbital plate and dental pad would also indicate improved utilization of calcium and phosphorus due to the ingestion of the magnesium supplements. The differences in the ash values were not due to differences in growth because C–170 and C–173 gained 360 and 256 pounds during the first 300 days of age while C–172, C–174, C–175 and C–176 each gained 376, 398, 451 and 353 pounds respectively during the same period. The rapid growth of C–175 probably accounts for the subnormal plasma calcium values toward the end of the experiment.

Our results indicate that some magnesium compounds have a vitamin D sparing effect. This sparing action may be due to increased solubility of calcium in the intestines since Forbes (15) reported that magnesium increased the solubility of calcium *in vitro*. It may be inferred that magnesium may be substituted for calcium in the excreta and by this means promote calcium utilization. The mechanism of the magnesium action may also be due to its effect upon phosphatase activity as suggested by von Euler and Rydbom (2) and Kay (3). Hommerberg (16) reported that with low concentrations of phosphatase, a certain amount of magnesium was necessary to activate the enzyme. He also stated that the phosphoric acid esters seemed to decompose spontaneously under the influence of magnesium.

From the fact that these magnesium compounds exerted a definite vitamin D sparing effect, it may be indicated that the magnesium content of feeds should be taken into consideration when evaluating their antirachitic potency. Legume hays and corn silage may exert a greater antirachitic influence than their vitamin D content would indicate.

SUMMARY AND CONCLUSIONS

1. The addition of magnesium carbonate to a rachitogenic ration failed to prevent the manifestation of clinical rickets in calves.

2. The addition of magnesium carbonate to the ration of C–89, after the onset of rickets, caused an increase in the plasma calcium and promoted the calcification of a fractured vertebra. Four pounds of wheat straw furnished the only source of vitamin D.

3. The rachitogenic ration fed to calves C–170 and C–173 supplemented with 5 cc. of cod liver oil per day did not protect these calves from rickets.

4. The addition of one per cent—on the dry-matter basis—of magnesium carbonate to the rations of calves C–172 and C–175 prevented the clinical

symptoms of rickets to 10.5 months of age. C–172 had normal plasma calcium and inorganic phosphorus values, while C–175 had normal inorganic phosphorus values and a few subnormal terminal calcium values during this period.

5. When the same level of magnesium oxide was added to the rations of C–174 and C–176, no clinical evidence of rickets was observed. The plasma calcium and inorganic phosphorus values were normal up to 20 months of age for C–174 and to 10.5 months of age for C–176. C–172 which received magnesium oxide in place of magnesium carbonate after 11 months of age had normal calcium and inorganic phosphorus values to 20 months of age.

6. The ash and mineral values of the bones indicated better calcium and phosphorus utilization due to the ingestion of magnesium supplements.

7. The results of this experiment indicate that the magnesium content of feeds for dairy cattle may contribute to their antirachitic effect.

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THE EFFECT OF SEASON OF THE YEAR AND ADVANCING LACTATION UPON MILK YIELD OF JERSEY COWS

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INTRODUCTION

Advancing lactation long has been observed to decrease daily milk yield of dairy cows, but the seasonal influence upon daily milk yield has been studied little, mainly for lack of a satisfactory method of analysis. Such a method was applied recently at the Florida station, in a study of variations in the butterfat tests of Jersey milk (3). The same method has been applied to an analysis of daily milk yields.

THE STATION HERD AND ITS ENVIRONMENT

The Florida station dairy herd earlier consisted of both registered and high grade Jersey cows, the majority of which were bred and raised on the farm. As described recently (3), the herd is located in the middle of the north end of the Florida peninsula, under an environment which varies less than does that of many dairy regions, due to proximity of large bodies of water and the general direction of the prevailing winds. Feeding practises have been relatively uniform over the period of years for which records were studied (1917 to 1932, inclusive), except for modifications in mineral content brought about mainly by inclusion of bone-meal in the concentrates. The rations and feeding practises followed were described in Florida station technical bulletin 262.

REVIEW OF LITERATURE

The influence of the season of freshening on total milk yield has been the object of a number of investigations. McDowell (13) assembled records of 64 cow testing associations between 1910 and 1920, comprising 10,870 cow-years. He found that the fall-fresh cows were the highest producers, followed by those calving during winter, summer and spring. Cannon (4) tabulated 68,000 records of Iowa dairy herd improvement association cows of all breeds for the years 1925 to 1930, and noted that the cows fresh during November produced the most milk, and those fresh during June yielded the least milk during the year. Wylie (18) analyzed 2,900 Jersey Register of Merit records completed in the United States in 1921, and noted that those Jerseys fresh in July produced the most, and those calving in November were the next highest in milk production. The group of cows that calved during August were lowest in milk yields.

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Hogstrom (12), using 891 lactations mainly by Ayrshire and grade Ayrshire cows in Sweden, observed the highest milk yields among cows calving in August, and the lowest in the group fresh in November. Headley (11), in Nevada, studied 124 lactations by Holstein cows fed uniformly throughout the year, and noted that the highest and lowest yielding groups freshened in winter and in September, respectively. Hammond and Sanders (10) found in England that the highest milk yields were obtained from cows calving in the autumn, and the minimum yields from those fresh in July, based on lactation records of four recording societies, with animals of eight breeds. Sanders (14) also found the highest milk production with cows that calved during October, November and December.

The decline in rate of secretion due to advancing lactation has been observed rather widely. Eckles (7) points out that the second month in lactation is the highest in milk production. From this point to the eighth month the decline is gradual, but it is more rapid from the ninth to twelfth months for cows calving annually. Cows not bred to calve within the year, as sometimes is practised on official test, do not show as marked a decline after the eighth month, due to delayed breeding. Carlyle and Woll (5) found on the average, an eight per cent decrease in milk yield for each month during the progress of the lactation period, in a study involving five breeds.

Woll (17) studied the lactations of 323 cows of three breeds, and found that the monthly decline in average daily milk yield was 2.3 pounds between the first and eighth months. From the ninth to the twelfth months, the decline was at the rate of 3.3 pounds, or a 43 per cent greater rate of decline in the daily milk production near the close of the lactation. Beach (2) showed the average monthly decrease from the first to the eighth months to be 6.5 per cent, but double that rate from the eighth to the tenth month after calving. Turner (16) studied 3,215 Guernsey Advanced Registry lactation records, and found the average daily production to be 34.3 pounds of milk in the second month; 23.7 pounds in the eighth month and 17.9 pounds in the twelfth month. With 305 Jersey Register of Merit records in Missouri, maximum daily milk yield was 30.0 pounds in the first month after calving; 20.6 pounds in the eighth month and 17.3 pounds the twelfth month. Ninety-five Holstein records in the station herd attained a maximum average daily milk yield of 40.3 pounds in the second month; 29.3 pounds in the eighth month and 20.1 pounds in the twelfth month. With cows under official testing conditions, breeding often is delayed in order that the inhibitory effect of advancing gestation may affect the record as little as possible. This may account for the lower rate of decline after the eighth month.

The seasonal influence on daily milk production has not been studied apart from other modifying factors. Many investigators have made obser-

vations similar to Farrington's (8), that milk yield increased when cows were changed from the stable onto spring pastures.

EXPERIMENTAL METHODS

Milk records of registered and high grade Jersey cows in the station dairy herd between 1917 and 1933 were assembled by ten-day periods, using the date of calving as the first day of the lactation. These were computed to uniform age basis, using the factors of Clark (6) for Jerseys milked twice daily. Lactations were grouped according to the month of freshening, and by seasons of the year, as defined previously for this latitude (1).

Statistical significance and the relationship of the variance arising from the advance in lactation and from milk yield at different seasons of the year, were determined by application of Fisher's "Z" test (9), as modified by Snedecor (15). The 144-cell arrangement of milk production by months, by groups of cows calving during each month of the year, allowed an even distribution of one factor throughout the data while making an analysis for the second factor. (Illustrated in Table 2).

PRESENTATION OF RESULTS

Season of freshening

The 319 lactations available for this study showed an average production per lactation of 5,262 pounds of milk, computed to maturity. The distribution of these lactation records according to season of calving, was reasonably uniform, as shown in Table 1. The differences in average milk yield between

TABLE 1

Average Milk Yield by Jersey Cows Calving at Different Seasons of the Year in Florida

SEASON	NUMBER OF LACTATIONS	AVERAGE MILK YIELD	PERCENTAGE OF AVERAGE LACTATION
Spring	74	4,863.6	92.42
Summer*	104	5,339.0	101.45
Autumn*	70	5,435.7	103.29
Winter	71	5,445.1	103.47
Average	319	5,262.6	100.00

* Because of the latitude and environment, "seasons" in Florida have been defined on the basis of the average dates of first and last killing frosts of winter (3 months), and on the duration of the rainy season in summer. On this basis, Summer includes June, July, August and September, and Autumn consists only of October and November.

groups, however, were not significant. It may be recalled in this connection that the seasonal variations in temperatures fluctuate within a narrow range in the Florida peninsula, because of its proximity to the Gulf of Mexico and the Atlantic Ocean.

TABLE 2

Effect of Advancing Lactation and of Season of the Year Upon Milk Yield in 319 Lactations by Jersey Cows at the Florida Agricultural Experiment Station, 1917 to 1932

MONTH AFTER CALVING	Average milk yield in pounds by 30-day periods for cows calving in the month of:												AVERAGE
	JAN.	FEB.	MARCH	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.	
First	707.7	583.0	665.9	655.8	644.1	699.6	704.4	647.9	639.1	686.8	663.2	678.4	664.6
Second	683.6	739.5	607.2	680.6	689.3	662.2	741.6	708.1	640.8	606.2	628.6	677.0	672.0
Third	649.0	622.7	690.5	553.0	642.3	653.5	583.7	667.2	652.6	544.1	522.4	560.3	611.8
Fourth	524.1	602.8	589.8	649.4	524.1	599.4	572.5	515.7	633.1	589.3	457.3	453.8	559.3
Fifth	437.1	485.8	548.8	564.9	633.4	507.5	555.9	504.5	462.0	573.8	481.2	394.8	512.5
Sixth	375.0	402.7	453.1	525.2	542.9	598.9	443.6	495.2	456.0	363.5	485.1	419.9	463.4
Seventh	386.8	362.7	374.2	437.4	515.5	513.3	534.3	394.0	424.7	385.4	275.9	456.9	421.8
Eighth	438.5	387.4	319.4	358.8	404.6	483.2	438.4	451.0	343.1	335.6	318.9	216.1	374.6
Ninth	178.5	392.7	332.7	260.9	321.7	373.3	387.6	332.1	387.2	276.6	244.8	254.7	311.9
Tenth	210.5	139.3	324.5	286.1	223.7	278.8	276.5	273.1	245.1	272.7	169.9	217.0	243.1
Eleventh	180.7	132.3	97.6	279.6	269.0	182.1	192.5	151.8	181.2	179.3	196.6	99.3	178.5
Twelfth	60.8	124.9	83.6	89.7	255.3	222.4	136.9	109.9	97.6	113.2	118.9	140.0	129.4
Average	402.7	414.7	423.9	445.1	472.2	481.2	464.0	437.5	430.2	410.5	380.2	380.7	5,142.9

ADVANCING LACTATION AND SEASONAL INFLUENCE ON MILK YIELD

The decline in milk production with advancing lactation and the rate of milk production in the different seasons of the year were measured by assembling the data for an analysis of variance (15). Individual milk yields were assembled by consecutive 30-day periods for the cows that freshened in the several calendar months. The calculated average milk yields thus do not represent *exact calendar months,* which vary from 28 to 31 days in length. Instead, they are 30-day periods for the groups of cows that freshened in these months, and in sufficient numbers that the records involved come very close to centering near the middle of each calendar month. Thus, the monthly milk yields were analyzed for both rate of production in consecutive months after calving, and also for calendar months throughout the year, irrespective of dates of calving. The results of this analysis are shown in Table 2.

The rate of decline with advancing lactation was measured with an even distribution of the seasonal influence throughout the data. Based on the previous month's milk production, the rates of decline in milk secretion after the second month in lactation were calculated to be 8.96, 8.58, 8.37, 9.58, 8.98, 11.33, 16.74, 22.06, 26.57 and 27.51 per cent, respectively. This shows a relatively uniform rate of decline from the second through the

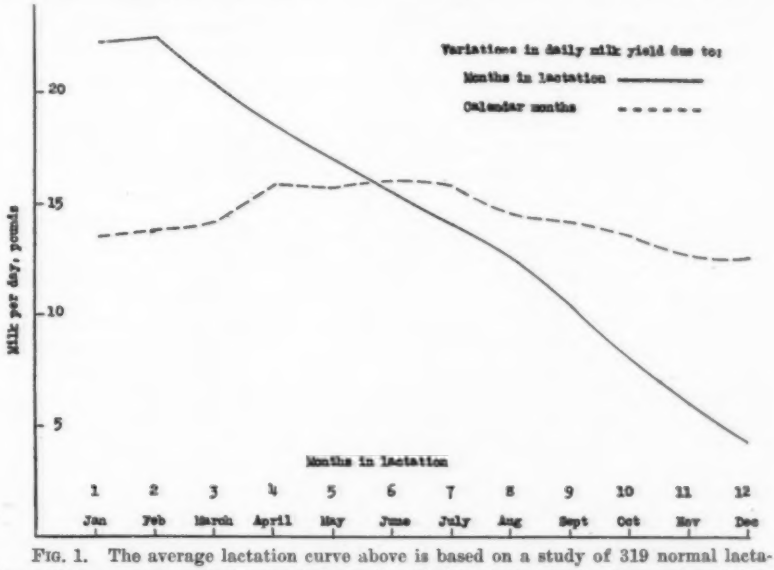

FIG. 1. The average lactation curve above is based on a study of 319 normal lactations of Jersey cows milked twice daily in the Florida station herd. Seasonal variation in milk production in the local environment at this latitude (30° N.) was only one-fourth the magnitude of the variation due to advancing lactation.

seventh month, and a progressively greater rate thereafter. This greater rate of decline in milk secretion in the later months of lactation has been shown by many investigators to be associated with the depressing influence of advancing stage of gestation.

Advancing stage of lactation was a far greater factor affecting milk yield than was season of the year, as seen in Figure 1, and in the analysis of variance in Table 3.

TABLE 3

Sources of Variance and Significance of Variations Affecting Monthly Milk Yields of Jersey Cows

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	"F" VALUE
Total	144 – 1 = 143	5,015,722.35		
Between months after calving	12 – 1 = 11	4,522,949.42	411,177.22	147.46
Between calendar months	12 – 1 = 11	145,389.65	13,217.24	4.74
Remainder	11 × 11 = 121	337,383.27	2,788.29	

The seasonal influence on milk production, irrespective of advancing lactation, acts within a relatively narrow range in the environment of Florida. The low average monthly milk production occurred during November and December, and the high average monthly milk production in June. The change in rate of production was gradual between these points.

TABLE 4

The Relationship Between Mean Temperatures, Average Rainfall and Monthly Milk Production of Jersey Cows at the Florida Station, 1917 to 1932

MONTH	MEAN TEMPERATURE			AVERAGE PRECIPITATION	AVERAGE MILK PRODUCTION
	Maximum	Minimum	Average		
	degrees	*degrees*	*degrees*	*inches*	*pounds*
January	68.7	46.5	57.6	3.15	402.7
February	71.7	48.7	60.2	2.83	414.7
March	75.4	52.8	64.1	3.62	423.9
April	81.4	57.4	69.4	3.23	445.1
May	86.5	62.5	74.5	3.46	472.2
June	89.7	68.8	79.25	6.96	481.2
July	90.6	70.8	80.7	9.07	464.0
August	90.7	70.8	80.75	6.27	437.5
September	89.0	69.1	79.05	5.36	430.2
October	82.9	61.2	72.05	2.56	410.5
November	74.6	51.4	63.0	1.94	380.2
December	69.5	47.6	58.55	2.59	380.7
Total				51.04	5,142.9

Milk yields began to decline before the highest mean temperature was attained early in the summer rainy season.

Moisture and warm temperatures favor the growth of pasture grasses. The relationship between grass growth and milk secretion was not considered. Agronomic data are necessary before this relationship can be studied. The average mean temperature, rainfall and milk yields by month, of Jersey cows in the Florida station herd for the years 1917 to 1932, inclusive, are shown in Table 4.

SUMMARY AND CONCLUSIONS

The relatively uniform environment of Florida was not conducive to significant differences in yearly milk production between groups of Jersey cows calving during the different seasons of the year.

Advancing stage of lactation, irrespective of season of the year, produced a relatively uniform rate of decline in monthly milk yield from the second through the seventh month in milk. The progressively greater rate of decline after the seventh month is associated with the inhibiting factor of advancing gestation.

The seasonal influence on milk yield, irrespective of advancing lactation, resulted in maximum rate of milk production during June, and the minimum during November and December. The peak of milk production was attained in advance of the mean maximum temperature early in the summer rainy season.

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Dr. Fred H. Hull advised with regard to methods of statistical analyses of the milk records reported herein.

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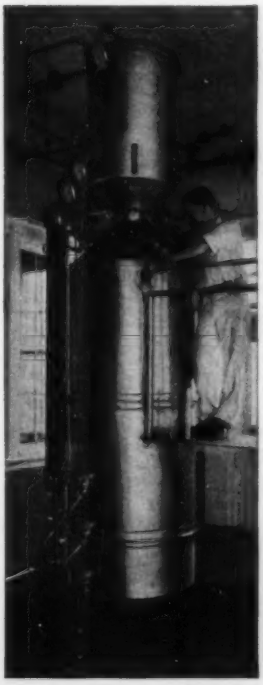

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